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Evaluation of Pain as a Discriminative Stimulus in the Rat

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EVALUATION OF PAIN AS A DISCRIMINATIVE
STIMULUS IN THE RAT

BY

STEPHEN MIKSIC

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
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IN
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Abstract

Intraperitoneal injection of phenyl-p-benzoquinone (PBQ) was used in three experiments to evaluate its action as a potential model for the study of "pain" processes. Pain is operationally defined as: behavior which would be interpreted as pain related in man, specific actions of analgesic drugs to reduce this behavior, and ability of other pain producing chemicals to substitute for the PBQ treatment. In the first experiment PBQ administration produced dose-dependent writhing in hooded rats. This behavior was blocked by the narcotic analgesic, morphine, also in a dose dependent manner. In the second experiment 20 subjects were trained to respond on a fixed ratio 10 schedule for food reinforcement. Two manipulanda were available for responding. One of the levers produced reinforcement only following an injection of PBQ, and responses on the other were only reinforced following a saline injection. Observation of performance over many trials indicated that PBQ was acting as a discriminative stimulus by significantly influencing lever choice prior to the delivery of reinforcement. However, the task was acquired with difficulty, was maintained at a low level of accuracy, and PBQ treatment was extremely toxic when given chronically. Procedural changes were incorporated in the third experiment to reduce or eliminate these problems.

The dose of PBQ was lowered from 1.25 mg/kg to 0.62 mg/kg, and the saline injection was omitted on non-PBQ trials. It was replaced by a handling treatment without injection. Twenty-six of a total of 49 subjects acquired the task, and a level of accuracy was maintained which permitted the application of experimental treatments. The subjects all met a criterion level of performance of 9 or fewer responses on the incorrect lever prior to the first 10 responses on the correct lever for 8 out of 10 consecutive trial days.

Discrimination of the PBQ injection was dose related, with an ED 50 of 0.21 mg/kg representing the average threshold. Neither decreased toxicity nor increased ease of acquisition resulted from the procedural changes. An injection of saline administered in place of PBQ elicited PBQ appropriate lever selection in 40% of the subjects; indicating that the discriminative stimulus was a compound, with PBQ irritation the primary component and probable irritation from the injection procedure a secondary component. Injection of histamine produced PBQ lever selection in a significant majority of the subjects tested. These data suggest that the discriminative property of PBQ treatment is its irritant quality since this action is common to both PBQ and histamine.

To further study the validity of PBQ discrimination as a model of subjectively experienced aversive stimuli

for investigation of pain related behavior, two analgesics and several other centrally acting drugs were administered with PBQ treatment on certain test trials. Both the narcotic analgesic morphine, and non-narcotic analgesic aspirin produced significant blockade of PBQ discrimination at doses which did not significantly affect response rate, and the blocking action was a significant linear function of ascending dose for morphine but not aspirin. Librium and haloperidol did not significantly affect PBQ discrimination at doses which severely depressed responding. Pentobarbital and amitriptyline interfered significantly with PBQ discrimination at one of several doses tested, but in a manner which excluded the effect as evidence of analgesic action. There was no significant dose-related blockade of discrimination by either drug, and the level of interference did not approach the effect of the two analgesic compounds tested.

It was concluded that PBQ treatment discrimination is based on both sensory and aversive components which are necessary to validate the task as a model of subjective pain processes. However, the problems of toxicity and performance reliability must be overcome before it can be used as a practical screening procedure for evaluating analgesic drug effects.

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General Introduction

Pain is an experience which includes both sensory and emotional components. When it functions as a discriminative stimulus, allowing us to avoid or escape present or impending physical harm, it is a healthy perceptual process. Pain which accompanies chronic illness or outlasts injury or disease is not useful, and can disrupt purposeful behavior.

Treatment for pathological pain is given primarily in the form of analgesic medication. Several experimental animal models presently exist for evaluating the potential analgesic activity of pharmacological and other treatment approaches. Many of these models are based on reactive-reflexive behavior which does not accurately measure pain as a subjective aversive experience. Operant procedures for assessing analgesic treatments are more sensitive to drug effects on subjective experience, but have concentrated on cutaneous stimulation and are subject to criticism on a number of methodological grounds. The following research is an investigation of a new model for the objective study of analgesic effects on subjectively experienced, chemically induced, interoceptive pain.

Background

Reactive-reflexive Methods for Measuring Analgesia

Thermal

The first use of heat to produce experimental pain was reported by Goldscheider in 1894. Since that time, the greatest impetus and inspiration for further investigation of thermal stimulation came from a study by Hardy, Wolfe and Goodell in 1940. First done in humans, the procedure involved application of radiant heat from a lamp or other source at a fixed duration. Intensity was increased until pain was reported. According to this test, a temperature of 45.5°centigrade was the reaction threshold in man, rat and guinea pig. The modification of this procedure by D'Amour and Smith (1941) is still one of the most widely used methods of producing experimental pain for assay of drug effects in animals. Light rays are focused on the tip of a rat's tail with steadily increasing intensity until it is withdrawn. Failure to move the tail after an exposure of a certain duration is indicative of analgesia.

Several methods for application of heat by direct contact were introduced by Andrews and Workman (1941), Wolfe and Macdonald (1944), Ben-Bassat et al. (1959), and Janssen et al. (1963). The production of a "skin-twitch"

in the dog through exposure to a radiant heat stimulus was studied by Andrews and Workman. This method has since been examined using rats (Tainter and Buchanan, 1949) and guinea pigs (Winder, 1947).

Paw licking followed by an escape response in mice placed on a heated surface (hot plate) was observed by Wolfe and Macdonald, who subsequently introduced this procedure for assaying the analgesic activity of drugs.

When the tail of a rodent, either mouse (Ben-Bassat et al. 1959), or rat (Janssen et al. 1963), is placed in a container holding a heated liquid; the latency of the tail-withdrawal response can also be used as a measure of pain sensitivity which can be modified by the administration of drugs.

With the exception of the "skin-twitch" procedure, these methods appear to be equally sensitive to the analgesic action of morphine, yielding an ED50 of approximately 3 mg/kg. The ED50 for reduction of "skin-twitch" is significantly greater.

Electrical

Application of electrical current to the skin surface on different areas of the body is frequently used as a model of pain. It is also criticized with equal or greater frequency. Application of shock is quite simple,

but control is particularly difficult.

Shock stimulation applied to the scotal sacs of rats was used by Thorpe (1946), and on the skin by Monje' and Ritter (1959). The presence or absence of a behavioral reaction at a certain voltage and amperage was the measure of sensitivity. Although the variability of the results was extreme in both studies, the authors felt that it is a useful method for assay of analgesic properties of new drugs.

Electrical stimulation of exposed tooth pulp has been used in cats (Scott and Tempel, 1963), guinea pigs (Wilder-Smith et al. 1963) and rabbits (Leaders and Keasling, 1962).

Shock has also been applied to the surface of tails of rats (Charpentier, 1962) and mice (Grewal, 1952) with an escape or vocalization response as the measure of nociceptive sensitivity. This procedure has been found effective by some investigators. Maxwell et al. (1961) were able to show parallel dose-response functions for morphine and codeine, with an ED50 of approximately 2 mg/kg for morphine. Other studies have found the method both insensitive and highly variable. Although response threshold was raised by 78% in the Charpentier study (1962), this was not a significant difference from baseline. McKenzie and Beechy (1962) could find only a barely detectable analgesic effect of 10 mg/kg morphine.

Even insertion of the electrodes beneath the skin of the tail failed to reduce threshold variability (Nilsen, 1961).

Reactivity to electrical stimulation introduced through a grid on which the animal stands, called the "flinch-jump" test, is another method proposed for assessing a drug's analgesic effect (Evans, 1961). Paw shock is delivered in both ascending and descending series. After each 0.1 second shock an animal is scored 1 (no response), 2 (flinch, or any reflexive movement of the body except that both rear paws do not leave the grid), or 3 (jump response, a reflexive movement in which both rear paws leave the grid). Each intensity of shock is usually delivered several times, and both flinch and jump threshold are calculated as the milliamperage at which an animal exhibited that response at least 5 out of 10 trials; as in Harvey et al. 1975. Evans (1961) found aspirin (100-750 mg/kg) and morphine (2-5 mg/kg) effective analgesics in this test, but the data does not allow a direct computation of ED 50.

Mechanical

In animal models the production of "pain" by deformation of tissue has been primarily limited to observations of the effect of application of gross pressure. These mostly involve paws or tails of rodents,

as seen in the early experiments of Haffner (1929) and Eddy (1932), which were popular prior to the thermal techniques of Hardy, Wolfe and Goodell (1940) mentioned earlier.

Vocalization has been the main indicant response, although it is frequently combined with "struggle" (Eagle and Carlson, 1950; Green et al. 1951). In these studies a sharp or blunt tipped metal rod was used, and pressure was measured by means of a scale, in grams; or in centimeters of mercury. The method that has received the most attention in recent years involves the application of increasing levels of pressure to a rodent's hind paw, which has been rendered hypersensitive by a subcutaneous injection of brewers yeast (Randall and Selitto, 1957). Both narcotic and salicylate analgesics effectively increase pressure tolerance in this test. Eagle and Carlson (1950) criticize the Randall-Selitto technique as cumbersome and difficult to administer because of the necessity of controlling the animal's paw position, and its insensitivity without the presence of inflammation.

Collier et al. (1961) prefer a more simplified procedure of applying artery clips to the toes of guinea pigs, and counting the number and frequency of resulting squeaks. Here, precise quantification of the pressure stimulus is not possible, but the resulting ED50 for morphine in reducing squeaks was comparable to the

Randall-Selitto method.

Each of the methods has its own specific advantages over the others, but they are approximately equal in sensitivity. A dose of three to five milligrams per kilogram of morphine given intraperitoneally or subcutaneously, or 180 to 750 milligrams per kilogram of aspirin orally, yields detectable analgesia by each of the procedures.

Chemical

Injection of chemicals that produce general or localized irritation through inflammation or other action is a frequently used analgesic assay procedure.

Phenyl-p-benzoquinone is a substance which causes centrally mediated abdominal muscle spasms in both rats and mice (Siegmund et al. 1957). These spasms are seen as a "writhing" response, or "extensions of the hind legs and twisting of the trunk". The writhing behavior is blocked by both morphine (ED50=1.15 mg/kg subcutaneously) and aspirin (ED50=160 mg/kg orally). Local anesthetics can block the writhing response (Vanderwende and Margolin, 1956), but not anticholinergics or antispasmodics (Siegmund et al. 1957). The release of serotonin or histamine does not seem to be involved (Eckhardt et al. 1958).

Bradykinin has also been used as a writhing inducing agent in similar experiments (Emele and Shanaman, 1963) as well as acetic acid (Niemgeers et al. 1975; Kokka and Fairhurst, 1977) and sodium-iodomethamate (Vander Wende and Margolin, 1956).

Recently, ethacrinic acid; a sulfhydryl reagent, was found to elicit a phenyl-quinone-like writhing syndrome that is antagonized by morphine (ED₅₀=0.42 mg/kg subcutaneously), aspirin (ED₅₀=88 mg/kg orally), and imipramine (ED₅₀=5.2 mg/kg orally) (Jaques, 1977).

This method appears to be more sensitive to analgesic effects of the test drugs. The dose of morphine needed to produce an ED₅₀ reduction in writhing is lower than with comparable doses of phenyl-quinone, and the writhing is also blocked by other psychotropic and non-steroidal anti-inflammatory drugs (Jaques, 1977).

Summary of Reactive-reflexive Analgesic Assay Techniques

Each of the foregoing nociception-inducing stimulus modes and techniques for their application and subsequent measurement of nociceptive responses, has individual advantages and disadvantages. They must be compared within a stimulus category because the separate modes of stimulation represent separate "pain" qualities. With regard to sensitivity to the effects of narcotic and non-narcotic analgesics, the stimuli may be compared.

Thermal, electrical, mechanical , and chemical modalities are all sensitive to analgesic effects of narcotics, but the chemical methods are the only procedures which are optimally sensitive to both narcotic and non-narcotic analgesics. This is probably due to inflammation as the source of nociceptive stimulation.

A common failing of all of the procedures concerns the nature of the response indicative of the presence, absence, or degree of "pain" affected by the drug treatment. All of the responses are primarily reflexive in nature. Thus, they produce data about reactivity rather than "pain" sensitivity (Winter, 1965). It is likely that subjective "pain" is already at a high level when the defensive reflex occurs (Goetzl et al. 1943; Miller, 1948; Beecher, 1957). Benjamin (1958) suggests that all reflexive "pain" indicators are actually side-effects of pain, and that an ideal analgesic should eliminate pain without affecting reflex activity. Therefore, the use of such behavior as "pain" indicators is undesirable. Most of the procedures also focus on cutaneous application of the stimulus, the only exception being the chemical approaches.

An attribute of pain evaluation that has been neglected by these procedures is subjective report. A more realistic evaluation of the effect of analgesic treatments on the perception of nociceptive stimulation

should provide a quantitative measure of the subjectively experienced aversive stimuli. Attempts to develop such an animal model for evaluation of analgesic treatments can be seen in studies utilizing procedures based on Skinnerian, operant tasks.

Operant Methods for Evaluation of Analgesics

In 1954, Hill et al. studied the effect of morphine on the conditioned emotional response (CER). An auditory stimulus paired with unavoidable shock in one situation, normally suppresses food reinforced bar pressing by a trained animal upon later exposure to the stimulus. This behavioral suppression was blocked by morphine given subcutaneously at four to eight milligrams per kilogram of body weight. Other investigators have found that morphine's anti-suppression effect is abolished in highly trained animals (Domino et al. 1963), and it is ineffective in doses which do not produce overall suppression of behavior (Launer, 1963). Launer (1963) and others (Tenen, 1967) have since identified anti-anxiety drugs of the benzodiazepine class as most effective in blocking the CER. Thus, drug effects on CER performance are not considered to be directly related to analgesic activity.

According to a different paradigm, subjects are exposed to electric shocks of increasing magnitude through a grid floor. The shock increments can be slowed or reversed by engaging in operant behavior such as lever pressing. The method is called "shock titration". The dependent measure is the maximum, or ceiling shock level tolerated by the subjects before lever pressing begins (Weiss and Laties, 1961). Salicylates (as in aspirin) and morphine both raised the level of shock tolerated by rats before avoidance lever pressing began. The dose of aspirin was 125 to 250 mg/kg orally, and 2.5 mg/kg morphine subcutaneously. In a similar procedure Mc Connell (1962) could not distinguish between effects of morphine and the benzodiazepine anxiolytic chlordiazepoxide. This is because substances which effect response rate through either peripheral or central action may not be distinguished from analgesic drugs. Benzodiazepine catalepsy may appear as an analgesic effect since response rate is retarded, leading to an increased level of shock being delivered. However, Malis (1962) reported differences between opiates and salicylates on the one hand, and pentobarbital and chlorpromazine on the other.

These findings indicate that the method has not always yielded consistent results, and is prone to false positive "analgesia" indications for drugs of different

classes. Also, whether or not a drug acts as an analgesic in this procedure depends on the schedule of shock delivery. Morphine and salicylates increase tolerated shock levels more if the shock is delivered every two seconds than if it is delivered every ten seconds (Weiss and Laties, 1961; Dykstra and Mc Millan, 1977). Dykstra and Mc Millan compared several analgesics, including morphine and pentazocine, with drugs not considered clinically as analgesics; such as amphetamine and diazepam. All of these drugs caused an increase in the median tolerated shock level when administered in the titration procedure. The authors did find a qualitative difference between morphine and the other drugs in that the maximum tolerated shock intensity was increased by morphine (3.0 mg/kg), while amphetamine (1.0 mg/kg) and diazepam (3.0 mg/kg) caused decreases in responding at the lowest shock intensities, resulting in the increased median shock levels.

Criticisms of this method include the confounding of a drug's effect on response rate with its analgesic properties, and the use of shock as the aversive stimulus. Shock intensity is extremely difficult to control because of changes in subject's resistance and conductivity at electrical contact points. In addition, the shock sensation may become a conditional stimulus for avoidance. A drug's effect could then be due to changes resulting

from learning, or effects on tactile sensitivity, as well as on central antinociceptive activity.

Milligan and Kallman (1963) were the first to use an aversive stimulus as a discriminative cue. By using shock as a cue for availability of reward they hoped to prevent it from acquiring conditional avoidance properties at low intensities. Rats were rewarded for lever pressing if responses followed a shock presentation within a limited period. This is a "go no-go" paradigm in which the presence of the aversive stimulus underlies the dependent measure of a go or no-go response. Morphine dose and shock level were varied. Morphine administration enabled the subjects to perform better at higher shock intensities. However, many more responses occurred outside of the correct response period. In this case a stimulant or disinhibiting action of a drug interferes with the subjects' ability to perform the task. Also, the problems inherent in the use of shock as the aversive stimulus are present. Since detection of the presence of shock was the positive discriminative stimulus for "when" to respond, the subject is likely to become sensitive to electrical stimulation whether or not it is perceived as aversive. The shock is always paired with reinforcement during training, and this could result in its acquiring secondary reinforcing properties and corresponding reduction in perceived aversiveness.

Summary of Operant Methods

Present operant behavioral methods for assessing analgesic properties of drugs have focused on shock as the aversive stimulus, and have used tasks where analgesic and gross response rate effects may become confounded. What is learned may not be reflected in what is measured so that interpretation of a drug's effect on the observed behavior is inaccurate. Seldom have the same authors compared the effect of a drug on both operant and reflexive behavior induced by an aversive stimulus. An effective operant approach would ideally be able to separate analgesic properties of a drug from its effects on response rate. At the same time, it would not utilize a relationship between the aversive stimulus and reward or punishment that could alter the perceived aversive qualities of the stimulation. A modified discriminative task similar to that of Milligan and Kallman could avoid these criticisms.

The Proposed Model

Aversive stimulation is readily discriminated by humans in most situations. We usually describe pain both in terms of its presence or absence, and in terms of its intensity. The use of peripherally induced aversive stimulation as a discriminative cue in laboratory animals provides the opportunity for objective measurement of a subjective state. Milligan and Kallman attempted this, but in a task which did not allow for a clear interpretation. If the subjects were given a choice of "where" to respond, rather than "when" to respond, several improvements in the Milligan and Kallman procedure would result.

According to this procedure, a subject is confronted with two manipulanda, and a single receptacle for delivery of reward. The presence of the aversive stimulus is the cue for reinforcement of responses on one lever, and the absence of the stimulus is the cue for availability of reward for responses at the other lever. A fixed ratio schedule of reinforcement coupled with a counterbalanced treatment schedule allows the observer to determine when, how accurately, and how quickly the task is learned. All responses which occur prior to reinforcement are based on the subject's expectancy of location of availability of reward. The discriminative stimulus can be seen to have gained control of location of responding when the first

non-reinforced responses on successive daily trials occur consistently on the appropriate lever, even when the two discriminative treatments follow a randomized or counterbalanced order.

The technique has been used to determine subjectively discriminable effects of internal, drug induced stimuli, including narcotic analgesics (Colpaert et al. 1975a; Shannon and Holtzman, 1976; Rosecrans et al. 1973; Lal et al. 1977), aspirin (Wiessman, 1976), and certain peripherally acting drugs (Colpaert et al. 1975b). Just as external auditory or visual stimuli can serve as discriminable stimuli, internally produced effects of stimulants, depressants, and narcotics can serve a similar function. Learning a response selection based on drug cues occurs at about the same rate as for a discrimination based on external, physical stimuli (Overton, 1971).

Generalization between drugs of similar classes, and within the same drug at different doses, parallel similar manipulations of external discriminative stimuli (Barry, 1974).

The use of an internal, chemically induced aversive stimulus in the above procedure could provide valuable information about drug effects on the discriminability of the stimulus, and basis of the discrimination. By using a two choice discriminative task the response rate and analgesic effects of a particular drug could be separated.

The choice of the "pain" appropriate lever as opposed to the "non-pain" lever, provides an assessment of analgesic efficacy when a drug is used to challenge the aversive stimulus. That the basis of the discrimination is the aversive quality of the stimulus, rather than its physical sensory characteristics, can be established by administering a known analgesic in conjunction with the aversive stimulus. If the analgesic does not interfere with sensory qualities, as with narcotic analgesics, but causes the subjects to respond on the "non-pain" lever, this is an indication that the discrimination is based on the subjective aversiveness of the stimulus and not on its purely sensory qualities. In addition, the behavioral toxicity of the drug will be seen in the overall response rate, no matter which response is chosen. The qualitative and response rate effects of the drug are independently measured by this procedure. The dose-response function for analgesic activity can be expressed by the proportion of subjects responding on the "pain" appropriate lever at each dose administered. There is no opportunity for the subjects to become sensitized to lower levels of the stimulus since this is not differentially rewarded by an opportunity to avoid the stimulus. The aversive stimulus is only paired with availability of reward on fifty percent of the trials, and is thus less likely to incur alterations in aversiveness as a result of secondary

reinforcement effects.

In summary, the method used in these experiments utilized food deprived laboratory animals who were taught to perform one response following injection of a chemical irritant, and another response when either saline or a handling treatment was administered. Discrimination of the stimuli produced by this chemical was challenged with several pharmacological treatments. This provided data on the ability of the subjects to act on the basis of discrimination of an aversive stimulus, and the ability of different drugs to interfere with, or substitute for, the stimulus. Administration of known analgesics provided information on the basis of the discrimination.

Experimental Treatments

Assay of phenyl-p-benzoquinone as a pain producing agent.

An injection 2-phenyl-1, 4-benzoquinone (PBQ) was used as the means of inducing noxious stimulation. In the first experiment the ability of PBQ to produce a behavioral reaction in rats, that could be quantified and would be reduced in a dose-dependent manner by a known narcotic analgesic, was studied. In the second and third experiments an injection of PBQ was used to produce a discriminative stimulus to be evaluated as a subjective,

painful experience by challenging the stimulus with established analgesics and non-analgesics. PBQ was selected as the means of inducing a noxious state because it is a potent writhing inducing agent (Vander Wende and Margolin, 1956; Siegmund et al. 1957), and this behavior is blocked by narcotic and non-narcotic analgesics (Siegmund et al. 1957).

Control treatments

In order to control for cutaneous stimulation and PBQ volume effects that could play a role in writhing induced by PBQ, saline in a volume equal to each PBQ treatment was injected and observed for its ability to produce a writhing response. Both saline injection and needle insertion were observed for their ability to substitute for a PBQ injection in the discriminative task. In addition, haloperidol was administered to determine whether non-specific central nervous system stimulation could substitute for PBQ. Haloperidol was chosen because it has a long onset of action that provided for a large temporal separation between injection and test (120 minutes), and has no known analgesic or intrinsic pain producing properties.

Blockade of PBQ-induced behavior

Morphine was selected as the prototypical analgesic for investigating the basis of PBQ writhing and discrimination. Effective morphine blockade of PBQ writhing and discrimination would suggest that these actions of PBQ are due to its ability to act as a painful stimulus. Blockade of morphine's effect by the narcotic antagonist, naloxone, was investigated to determine whether this action was narcotic specific. When this was established, other drug treatments were observed for their ability to block, or interfere with, PBQ-induced discrimination. Aspirin and morphine are the two drugs most frequently used for relief of pain (Houde et al. 1965), and are used in most investigations of the basic mechanisms of analgesia (Mayer and Price, 1976; Fuccella et al. 1977). Several other centrally acting drugs having one or more actions in common with morphine were also tested for their effect on choice performance under the PBQ condition of the discriminative task. These are listed below.

Pentobarbital was chosen because it is a potent barbiturate central nervous system depressant without clinical analgesic efficacy, and may even produce pain in certain patients (Goodman and Gilman, 1975a). Both positive (Weiss and Laties, 1961) and negative (Malis, 1962) analgesic indications were found for this

drug using shock titration. Chlordiazepoxide is an antianxiety compound of the benzodiazepine class, and a potent muscle relaxant (Goodman and Gilman, 1975b).

Mc Connell (1962) found that chlordiazepoxide may act similar to morphine in shock titration. Amitriptyline is a tri-cyclic anti-depressant that is not generally considered to possess analgesic activity, but may reduce pain-related affective disturbances (Goodman and Gilman, 1975c; Halpern, 1974). Haloperidol can significantly increase the analgesic potency of morphine (Tulunay et al. 1976), presumably due to its ability to block the access of dopamine to post-synaptic receptor sites (Goodman and Gilman, 1975d). Haloperidol is primarily used as an anti-psychotic medication, and appears devoid of any analgesic properties of its own (Goodman and Gilman, 1975d). Morphine is known to possess anti-anxiety and anti-depressant activity associated with euphoria, central nervous system depression, and blocks dopamine receptors in conjunction with analgesia (Goodman and Gilman, 1975e).

Generalization of PEO discrimination to other chemical irritants

Histamine is a potent naturally occurring physiological agent that is released from cells upon

injury (Lewis, 1927) It is a powerful smooth muscle stimulant and sub-dermal irritant (Goodman and Gilman, 1975f).

This drug was administered to determine the specificity of the stimulus produced by PBQ. Its substitution for PBQ in the discriminative task would be a further indication that the stimulus control is based on a subjective "painful" state.

Summary

The significance of this research lies in the use of an operant technique which provides for quantification of a subjectively perceived stimulus. A chemical irritant was observed for its ability to support discriminative responding in rats. Known analgesic drugs were used to demonstrate that a subjective "painful" experience, and not just peripheral stimulation, formed the basis of the discrimination. Several centrally active, non-analgesic drugs were tested for their effects on the discrimination to evaluate its specificity and adequacy as a model for study of analgesic treatments. A peripheral pain producing chemical was tested for generalization with the discriminative stimuli produced by PBQ; the experimental aversive stimulus.

Experiment 1

Assessment of Phenyl-p-benzoquinone induced Writhing and its Antagonism by Morphine

Introduction

Prior to evaluation of PBQ as a discriminative stimulus, its ability to induce writhing in rats and the effect of a narcotic analgesic on this behavior was investigated. Several doses of PBQ were administered alone and following pretreatment with morphine, to establish dose-dependent effects of PBQ induced writhing, and morphine blockade of the writhing syndrome. This was done to verify PBQ activity as a model of aversive stimulation able to be reduced by a known analgesic.

Methods

Subjects

Twelve male, Long-Evans rats were obtained from Charles-River Breeding Company, Wilmington, Mass. At the beginning of the experiment they weighed 300 to 335 grams. All rats were housed singly in a thermostatically controlled environment with a 12 hour dark-light cycle. Food and water were available at all times in the subject's home cages.

Drugs

Phenyl-p-benzoquinone (PBQ) solution was prepared freshly each day by dissolving the appropriate weight in two milliliters of pure alcohol, and adding this solution to a volume of distilled water heated to 50°C.

Morphine sulphate solution was prepared as needed by dissolving morphine sulphate in physiological saline to the desired concentration. Saline was prepared at 0.9% by adding sodium chloride to distilled water, and was kept in quantity.

Behavioral observations

Immediately after injection with either PBQ or saline, each rat was placed in a clear plexiglas container and observed for frequency of writhing for 30 minutes. A writhe was defined as "intermittent contraction of the abdominal muscles with extension of the hind legs and twisting of the trunk", after Siegmund et al. (1957). The presence of all of these components was necessary for a response to be recorded as a writhe. An animal had to return to a normal posture before another writhe could be recorded. No more than three animals were observed by a single scorer at any one time. The observations were carried out in exactly the same manner when effects of morphine on PBQ writhing were observed. Morphine was administered 15 minutes prior to PBQ.

Experimental Procedure

To observe the effect of intraperitoneal injection of PBQ, the group of 12 rats was initially divided into two groups of six subjects. One group received 1.25 mg/kg PBQ, and 5.0 mg/kg was administered to the remaining subjects. The following day 10.0 mg/kg PBQ was administered to all 12 subjects. For the next two days, the corresponding volumes of physiological saline were injected to observe the effects of the PBQ vehicle. First, the 12 subjects were randomly divided into two groups of six. One group was given 1.25 ml/kg, and the second group 5.0 ml/kg. These were equivalent volumes to PBQ doses of 1.25 and 5.0 mg/kg. Two subjects from each of these groups (a total of four) were tested with 10.0 ml/kg saline the following day. The experimental design can be seen in Table 1.

Effect of morphine pretreatment

To determine the effect of a narcotic analgesic on PBQ induced behavior, morphine sulphate was injected I. P. 15 minutes prior to PBQ in a sequence of tests. Log doses of 1.25, 2.5 and 5.0 mg/kg were administered to all subjects in counterbalanced order, as seen in Table 2.

Effect of repeated PBQ administration

A dose of 10.0 mg/kg PBQ was administered to four

TABLE 1
Experimental Treatment Schedule for Assay of
PBQ-induced Writhing

Day	Subject	Dose	Drug
1	1 - 6	1.25 mg/kg	PBQ
	7 - 12	5.00 mg/kg	PBQ
2	1 - 6	10.00 mg/kg	PBQ
	7 - 12	10.00 mg/kg	PBQ
3	1 - 6	1.25 ml/kg	Saline
	7 - 12	5.00 ml/kg	Saline
4	3,6,9,12	10.00 ml/kg	Saline

TABLE 2

Dose¹ Schedule for Determination of the
Effect of Morphine on PBQ-induced Writhing

	DAY		
	1	2	3
1	1.25	2.50	5.00
2			
3			
4			
5	2.50	5.00	1.25
6			
7			
8			
9	5.00	1.25	2.50
10			
11			
12			

1 Mg/kg injected I.P. 30 minutes prior to the
Writhing observation period.

subjects for six successive days to investigate the possibility of tolerance to the behavioral effects of PBQ. The four subjects were randomly selected from the original group of 12 subjects.

Results and Discussion

Intraperitoneal injection of PBQ in doses of 1.25, 5.0 and 10.0 mg/kg produced dose dependent writhing. The data are presented in Table 3 and Figure 1. A linear regression analysis yielded a correlation coefficient of 0.998, and a significant analysis of variance value for the regression ($F, df1, 1=332, P < 0.035$). An analysis of variance performed on the mean frequency of writhing for each dose also produced a significant F value ($F, df1, 8=8.41, P < 0.01$). A followup Tukey test indicated that the mean writhing frequency induced by 10.0 mg/kg PBQ was significantly different from that produced by 1.25 mg/kg ($T df15=19, P < 0.01$). The only significant difference in writhing was between these two doses of PBQ. Although the writhing levels of 10 mg/kg compared to 5 mg/kg, and 5 mg/kg compared to 1.25 mg/kg appear quite large, the equally large variability in writhing frequency for individual subjects resulted in an inability to attain statistical significance between these groups with the low number of subjects utilized.

The data show that PBQ injection results in dose-dependent writhing in hooded rats. That the writhing response is due entirely to the action of PBQ, and not the injection procedure or fluid volume, is demonstrated by the failure of injection of similar volumes of

TABLE 3

A Comparison of Writhing Produced by PBQ or Saline

Drug	Dose ¹	N	Frequency of ² Writhing
PBQ	1.25	6	3 \pm 1.3
PBQ	5.00	6	13 \pm 6.6
PBQ	10.00	12	22 \pm 4.4
Saline	1.25	6	0 \pm 0
Saline	5.00	6	0 \pm 0
Saline	10.00	4	0 \pm 0

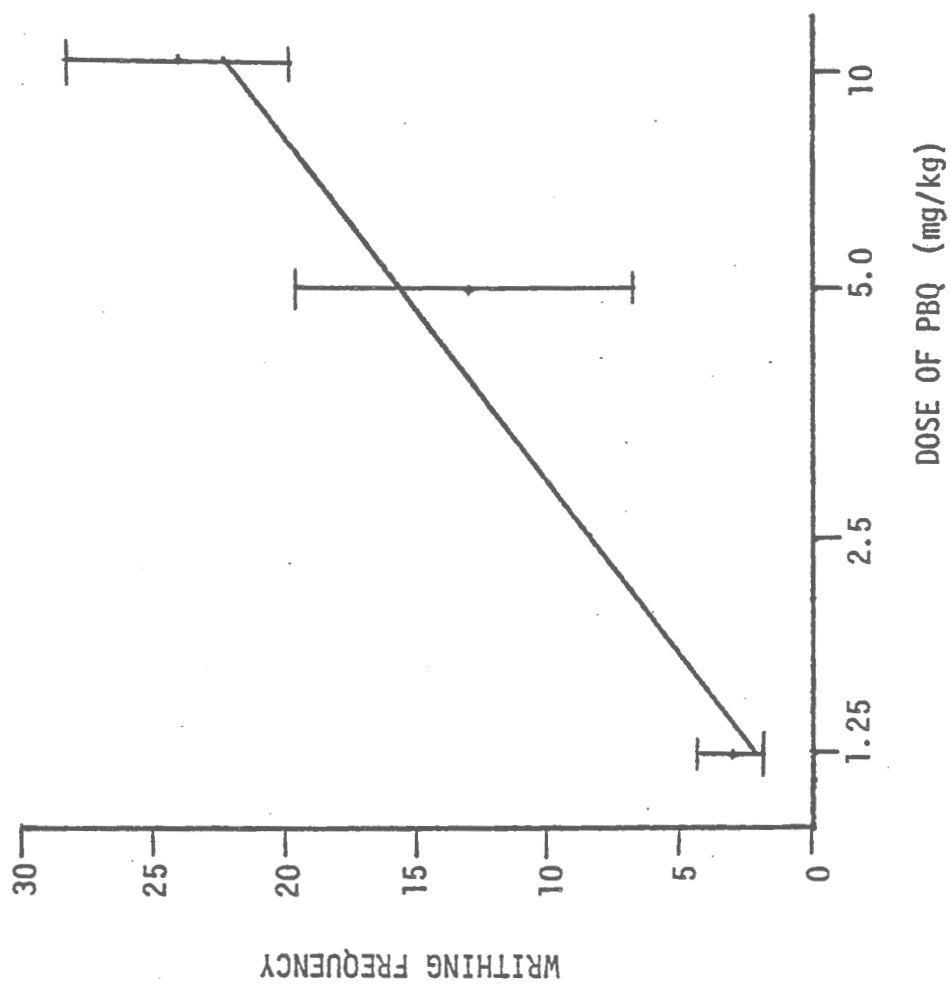
1 Mg/kg of PBQ, and ml/kg saline; both injected I.P. immediately prior to observation.

2 Mean \pm standard error for a 30 minute observation period.

Figure 1

Dose Response for PBQ - Induced Writhing in Hooded Rats

The Y axis represents writhing frequency. Mean writhing frequency \pm standard error is plotted for 6 subjects at 1.25 and 5.0 mg/kg doses of PBQ (given I.P. immediately before a 30 minute observation period) and 12 subjects at 10.0 mg/kg PBQ. PBQ doses are shown on the X axis.



physiological saline to induce any writhing whatsoever (see Table 3).

When morphine was administered 15 minutes prior to 10.0 mg/kg PBQ, dose-dependent blockade of the writhing is seen, as shown in Table 4 and Figure 2. The correlation coefficient for the computer plotted best fitting linear function was 0.99 for dose vs. mean writhing level on the X and Y axes, respectively. An analysis of variance performed on the regression was significant ($F_{df1,1}=176.4, P < 0.05$). An analysis of variance for comparison of mean writhing levels for the three doses of morphine was also significant ($F_{df2,18}=3.52, P < 0.05$). A Tukey followup analysis found a significant difference between doses of 5.0 mg/kg and 1.25 mg/kg only ($T_{df22}=15, P < 0.05$).

Writhing frequency remained at a high level when PBQ was injected once per day for 6 consecutive days (refer to Table 5 and Figure 3). The regression coefficient for comparison of total writhing frequency vs. days was 0.56. The analysis of variance for the regression was non-significant ($F_{df1,4}=14.8, P > 0.05$), as was an analysis of variance performed on the 6 mean daily writhing frequencies ($F_{df5,18}=0.57, P > 0.05$).

It was concluded that PBQ administration is effective in producing a behavior which may be interpreted as pain-related since a known analgesic, morphine, blocks the

TABLE 4
Effect of Morphine¹ on PBQ²-induced Writhing

Dose (mg/kg)	Frequency ₃ of Writhing
0	22 \pm 4.4
1.25	15 \pm 5.4
2.50	9 \pm 4.5
5.00	0 \pm 0

1 Injected I.P. 30 minutes prior to observation.

2 10 mg/kg I.P. immediately before observation.

3 Mean \pm standard error for a 30 minute observation period.
Each value represents 12 subjects.

Figure 2

Effect of Morphine on PBQ - Induced Writhing

PBQ - induced writhing was observed for 30 minutes after pretreatment with morphine. The mean \pm standard error of writhing frequency was plotted for each dose of morphine. Morphine doses were given I.P. 30 minutes prior to observation in all 12 subjects in counterbalanced order, and are shown on the X axis. Writhing frequency is shown on the Y axis.

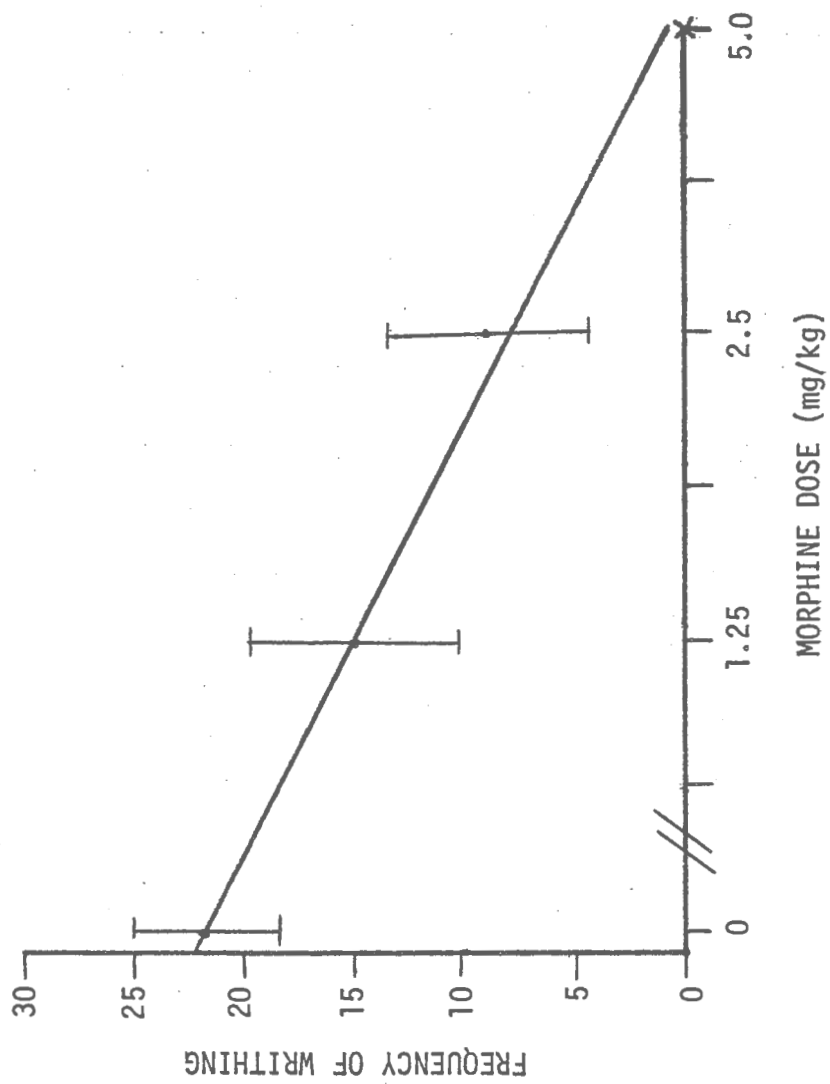


TABLE 5

PBQ¹-induced Writhing during Chronic Administration

Day	Frequency of Writhing ²
1	13.50 \pm 1.6
2	11.25 \pm 2.7
3	10.25 \pm 1.25
4	9.00 \pm 4.3
5	12.75 \pm 2.2
6	8.75 \pm 2.2

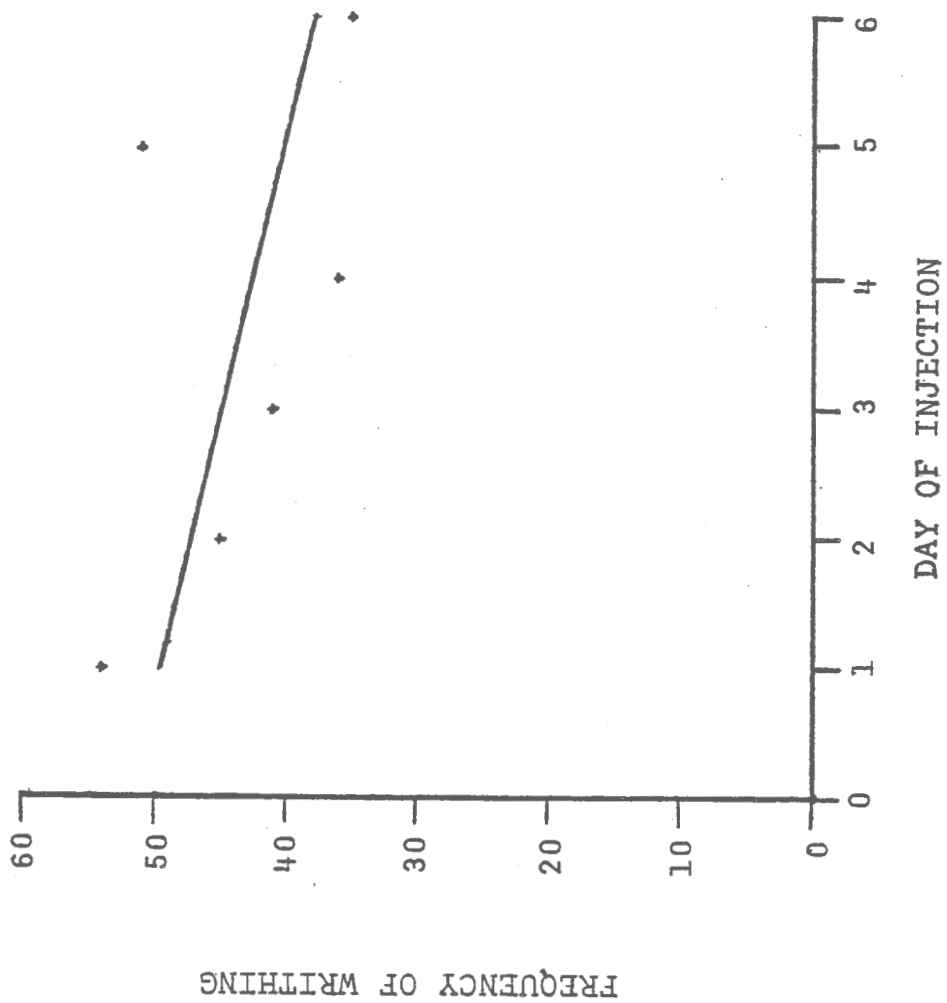
1 10 mg/kg I.P.

2 Mean \pm standard error for a 30 minute observation period.
N = 4 for each value.

Figure 3

Frequency of Writhing during Chronic PBQ Treatment

PBQ at 10.0 mg/kg was administered once per day for 6 days (X axis) to determine whether significant tolerance to PBQ writhing would develop. The total frequency of writhing (Y axis) is plotted for the group for each of the 30 minute observation periods.



writhe inducing action of PBQ in a dose-related manner. Based on this, it was concluded that PBQ would be an effective stimulus in a discriminative operant task. Maintenance of a discrimination of PBQ from an alternate treatment would provide for determination of the role of subjective aversive attributes of the stimulus as the basis of the discrimination, and as a model of pain processes. This was investigated in Experiments 2 and 3.

Experiment 2

Evaluation of PBQ Administration as a Discriminative Stimulus

Introduction

Following assessment of PBQ induced writhing and its blockade by morphine pretreatment, the ability of PBQ administration to serve as a discriminative stimulus was studied. In the first experiment PBQ and saline injections served as the two conditions to be discriminated.

Methods

Subjects

Male rats of the Long-Evans strain weighed 300 to 340 grams at the beginning of the experiment. The 20 subjects were housed as in Experiment 1. Water was available at all times in the home cages, but food was limited so that the animals were maintained at 80% of free feeding body weights.

Procedure

The subjects were trained to lever press for food (Noyes 45 mg pellets) on each of two levers mounted on one

wall of standard operant chambers. The food receptacle was located between the levers and was equidistant from each. Discrimination training and shaping of fixed ratio performance were carried out simultaneously. Responding on one of the two levers was reinforced after an injection of 1.25 mg/kg PBQ given 5 minutes before each trial. The opposite lever produced reinforcement following an injection of saline at the same injection-trial interval. Assignment of PBQ correct lever position for each subject was randomized, and PBQ or saline treatments were counterbalanced according to the following two sequences: PBQ - S - S - PBQ - S and PBQ - PBQ - S - PBQ - S - S. These sequences were presented in randomized order with the stipulation that no treatment was ever given more than twice in succession. Trials were carried out once a day at least 5 days per week, and were of 10 minutes duration. Total reinforcements, total incorrect responses, and incorrect responses occurring prior to the first reinforcement, were recorded for each subject for each trial.

Results and Discussion

PBQ toxicity

The experiment was carried out for a total of 90 trials. During this period 16 of the original 20 subjects were discontinued. Death was the cause in 6 subjects. The other 10 became ill, lost weight, and would not respond when placed in the operant chambers. This indicates that the dose of PBQ used for training was quite toxic, causing peritoneal inflammation and infection with swelling of the gut and loss of task performance in a majority of the subjects.

Discrimination performance

An analysis of the trial by trial data shows that 7 of the 20 subjects reached the level of performance that was considered indicative of reliable learning of the task. This is 8 out of 10 consecutive trials for which 9 or fewer responses occurred on the "wrong" lever prior to the first reinforcement on that trial. The mean number of trials for reaching this criterion was 49 ± 8 , with 20 being the least, and 80 the greatest number of trials required to reach the criterion. The mean number of trials correct for each 10 trials after reaching criterion was 6.5 ± 4 , indicating that maintenance of the task was relatively unstable. An examination of the data suggests that much

of this instability was due to behavioral toxicity of the PBQ, as the decline in accuracy generally preceded illness or death of the subject. However, response rates on PBQ and saline trials did not significantly differ when an average of trials 10 through 30 (the first 10 trials for each treatment) were taken for each subject and compared for the group by means of a dependent t test (PBQ=774±45, saline=803±68, $t_{df18}=0.59$, $P > 0.05$).

Analysis of the group data also shows that significant learning occurred for the group as a whole. A regression analysis of the number correct in each of the 9 blocks of 10 trials demonstrated that the increase in correct trials over days is a linear function for the group ($r=0.82$, $F_{df1,7}=14.8$, $P < 0.006$). For a presentation of the data see Figure 4.

Summary

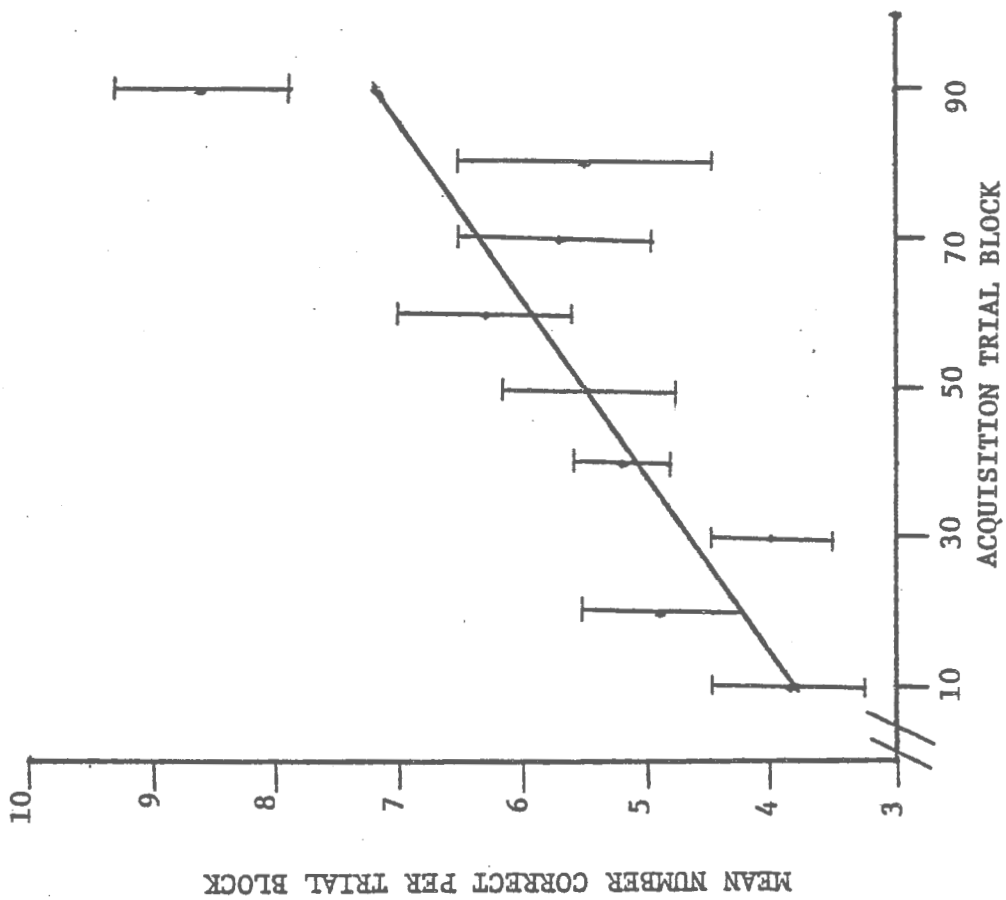
The data of this experiment show that PBQ administration can be discriminated from a saline injection, since seven subjects attained a rigorous criterion performance, and the group as a whole improved significantly in overall performance.

Although this is a conclusive demonstration that PBQ-saline discrimination can be acquired, the performance is not of sufficient reliability to provide a behavioral baseline for a model to evaluate analgesic treatments.

Figure 4

Acquisition of PBQ - Saline Discrimination

Performance during acquisition of PBQ - saline discrimination is presented as the mean \pm standard error of correct lever selections (Y axis) in each 10 day period (X axis) during the course of the experiment. One 10 minute trial was given per day, preceded by either a PBQ or saline injection (I.P.) 5 minutes before the trial. Correct lever selection was defined as 9 or fewer responses on the incorrect lever before the first 10 responses were completed on the lever appropriate for that day's injection treatment (either PBQ or saline).



Discrimination of centrally acting drugs, such as narcotics and barbiturates, is maintained at an extremely high level of accuracy (Colpaert et al. 1975; Lal et al. 1977). There are two likely explanations for the low level of accuracy in the present experiment. The first is tissue damage accompanied by loss of ability to detect the stimulus. This is equivalent to a constant, high level of irritation which develops with chronic PBQ treatment and prevents discrimination of the acute PBQ stimulus on those test trials. However, some subjects continued to perform without noticeable illness for long periods during which accuracy for discrimination fluctuated to a great degree; from 3 to 10 out of 10 correct in one instance of 20 consecutive trials. This may be explained by the existence of masking irritation which developed during certain periods and prevented accurate performance on PBQ or saline trials.

A second possible cause for difficulty in task acquisition is that the saline injection, in itself, may produce significant irritation making the PBQ - placebo discrimination difficult.

Two experimental task modifications were introduced in the third experiment to minimize detrimental effects of these possible complications and optimize discriminability of the PBQ and non-PBQ conditions. First, the dose of PBQ was lowered to 0.62 mg/kg. This was done in order to

reduce the chance of tissue damage and development of chronic irritation with repeated PBQ administration during training. Secondly, the saline treatment that was alternated with PBQ as the treatment to be discriminated, was discontinued. The advantage sought was increased distinctiveness of the PBQ from non-PBQ treatment. On the alternating non-PBQ trials the animals now received only a handling treatment. A major problem with this procedure was that the injection could become a significant component of the discrimination in addition to PBQ induced stimuli. This possibility was accepted for two reasons: 1.) When PBQ discrimination was attained to a high level, saline could be injected on certain test sessions to determine the role of the injection procedure in the discrimination 2.) If the injection procedure was a significant component of the discriminative stimulus, it could also be evaluated as a model of pain itself.

Experiment 3

Characterization of a Discrimination based on PBQ Treatment

Introduction

In Experiment 2 it was demonstrated that PBQ and saline administration will acquire differential stimulus control of behavior. This control was of a low degree of accuracy, possibly due to the similarity between PBQ and saline treatment. In this experiment conditions were optimized for discriminability of PBQ from the non-PBQ treatment. When a criterion for discriminative performance was achieved by each subject, several experimental and control treatments were applied on certain trials to determine the adequacy of PBQ discrimination as a model of pain for the study of analgesic treatments.

Methods

Subjects

A total of 49 male, Long-Evans rats were employed. They were housed as were the subjects in Experiment 2.

Procedure

Magazine training and shaping to respond on an FR10

schedule for food reinforcement in dual lever operant chambers was carried out as in Experiment 2.

Discrimination training was carried out as in Experiment 2 except that saline injections were not administered. The subjects were trained to discriminate a PBQ injection from handling without injection, according to the same schedule of counterbalanced treatments. The dose of PBQ was 0.62 mg/kg.

When a criterion was reached of not more than 9 responses on the incorrect lever for 8 out of 10 consecutive trials, the following experimental and control treatments were applied (for details of dose and injection-test interval for each drug see Table 6). The order of drug and dose testing was counterbalanced (for details see Appendix 1).

Control and generalization treatments. The ability of different treatments to produce PBQ appropriate lever selection was tested in the absence of PBQ. Saline was injected, or a needle inserted without injection, 5 minutes prior to testing to determine whether the injection procedure or vehicle volume would be perceived as PBQ treatment. On some test trials 0.62 and 0.16 mg/kg PBQ were administered for observation of dose-response effects. Haloperidol was injected on other test days to observe whether the effect of a centrally acting drug would be perceived as PBQ or non-PBQ treatment, to

establish the stimulus specificity of PBQ. In addition, a known chemical irritant (histamine) was injected intraperitoneally. This substance is reported to be a potent pain producing agent. Its ability to produce PBQ appropriate responding was investigated to determine if the subjective stimuli produced by the drug are similar to that of PBQ.

Experimental treatments. Morphine and aspirin were administered prior to PBQ treatment to determine whether narcotic and non-narcotic analgesics antagonize PBQ treatment discrimination. Other non-analgesic, centrally acting drugs were administered on certain PBQ trials to evaluate the specificity of antagonism of PBQ treatment discrimination.

Four subjects were administered 10 mg/kg PBQ at the conclusion of the experiment, and writhing behavior was observed for 30 minutes. This provided an indication of the effect of the chronically administered 0.62 mg/kg training dose on sensitivity to the PBQ action. The subjects each had at least 50 prior PBQ treatments.

Five additional subjects were injected with 1.0 ml/kg saline and observed for writhing for 30 minutes. If the injection procedure had become a conditional stimulus for PBQ treatment able to elicit writhing by virtue of pairings with PBQ treatment, this could be detected.

Results and Discussion

Task acquisition and PBQ toxicity

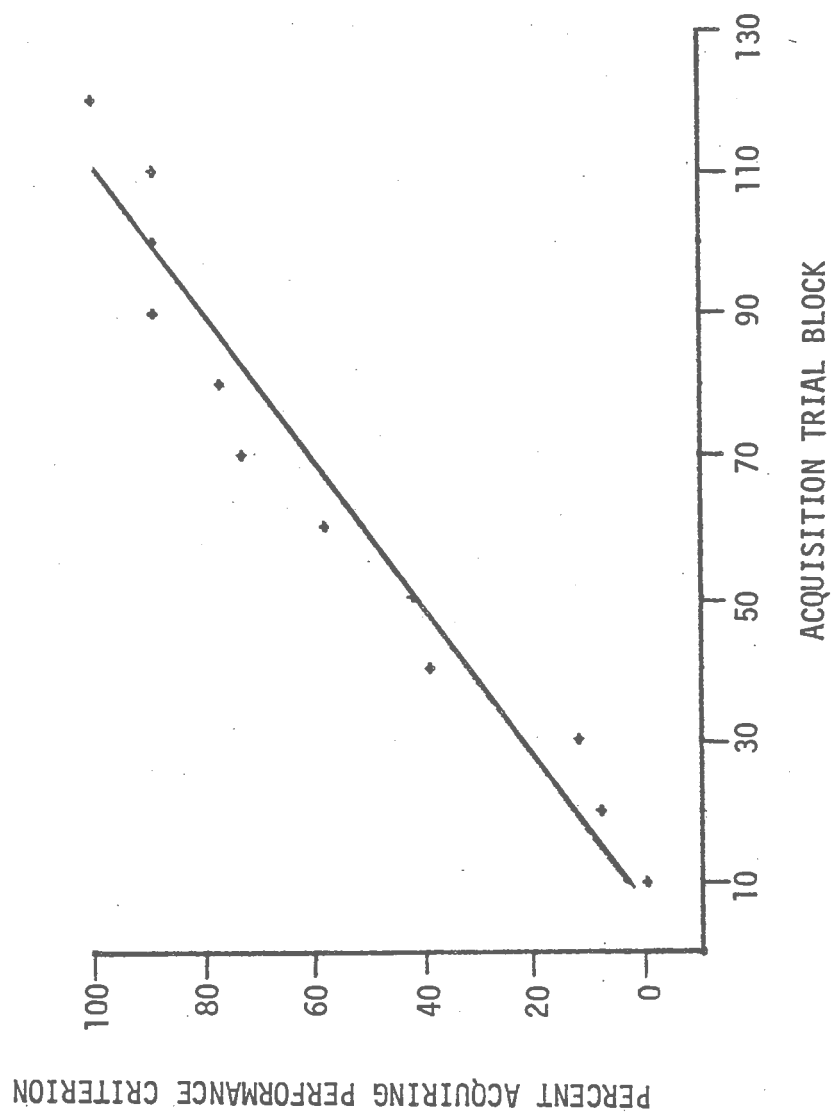
Of the 49 subjects utilized, 26 completed the experiment, 7 died during the course of the study, and 16 were discontinued due to illness which interfered with performance. Criterion performance was reached by 26 of the 49 subjects in a mean of 58 ± 6 trials. The fewest number of trials required for attaining the criterion level was 16, and 116 trials the most. As in Experiment 2, there was no significant difference between PBQ and non-PBQ trials for mean response rate levels (mean for saline trials 10 through 20 = 774 ± 40 , and 769 ± 33 for PBQ trials 10 through 20 for the 26 subjects reaching criterion, $t_{df52} = 0.20$, $P > 0.05$).

Comparing these data to those of Experiment 2, it is clear that a PBQ injection is discriminated from a non-PBQ treatment. Learning of the task took place at a comparable rate in both experiments (49 ± 8 trials to criterion in Experiment 2, and 58 ± 6 trials in Experiment 3), although the dose of PBQ used in Experiment 3 was half of that used in Experiment 2. The acquisition data are presented in Figure 5. A higher proportion of the total number of subjects used actually acquired the discrimination in Experiment 3 (26/49 or 53%) as compared

Figure 5

Acquisition of PBQ Treatment Discrimination

The cumulative percentage of the total number of subjects (26) who acquired the PBQ - No treatment discrimination in Experiment 3 is plotted for each block of 10 trials until the final subject attained the criterion.



to Experiment 2 (7/20 or 35%). This difference was not significant as determined by χ^2 analysis (χ^2 df1=1.89, $P > 0.05$). Also, only 5 of the 20 animals who originally began Experiment 3 completed more than the initial 90 trials. This indicates that the discrimination of Experiment 3 can be acquired by more subjects than that of Experiment 2, but it is not learned faster, nor is the PBQ treatment less toxic even though the dose is half as large.

PBQ dose-response and generalization tests

When criterion performance was reached, doses of 0, 0.16, or 0.62 mg/kg of PBQ were administered on certain test trials to 10 subjects at each dose according to the counterbalanced treatment schedule seen in Appendix 1. The data, shown in Table 7 and Figure 6, demonstrate that the dose response for PBQ discrimination in this experiment is linear ($r=0.983$, F df1,1=361, $P < 0.05$). All percentage data were transformed to a common logarithmic value prior to regression and analysis of variance computation. The ED50 for PBQ discrimination was 0.26 mg/kg. Administration of a saline injection to 20 subjects produced PBQ treatment responding in 8 animals, or 40% of the total number tested, as shown in Table 8. This proportion was significantly different from that found either for no-treatment (χ^2 df1=10, $P < 0.01$) or for

TABLE 6

Summary of Test Treatments for Experiment 3

Drug	Dose ¹	Lever Choice ² PBQ/Total	Response	Treatment-Test Interval (min.)
			Rate Mean \pm S.E.	
None	----	0/20	774 \pm 40	5.0
Saline Injection	1.00	8/20	914 \pm 58	5.0
Saline Injection	1.00	6/12	997 \pm 93	30.0
Needle Insertion	----	5/10	816 \pm 81	5.0
PBQ	0.62	20/20	769 \pm 33	5.0
PBQ	0.62	9/12	999 \pm 99	30.0
PBQ	0.16	5/10	830 \pm 78	5.0
Histamine	1.25	6/6	807 \pm 65	5.0
Morphine +	10.00			30.0
PBQ	0.62	0/5	192 \pm 45	5.0
Morphine +	5.00			30.0
PBQ	0.62	1/5	752 \pm 136	5.0
Morphine +	1.25			30.0
PBQ	0.62	4/5	762 \pm 184	5.0
Morphine +	5.00			30.0
Naloxone +	1.25			10.0
PBQ	0.62	5/5	846 \pm 88	5.0
Amitriptyline +	40.00			60.0
PBQ	0.62	2/3*	171 \pm 85	5.0
Amitriptyline +	20.00			60.0
PBQ	0.62	5/6	585 \pm 160	5.0
Amitriptyline +	10.00			60.0
PBQ	0.62	3/6	650 \pm 36	5.0

TABLE 6 Continued

Drug	Dose ¹	Lever Choice ² PBQ/Total	Response	Treatment-Test Interval (min.)
			Rate Mean \pm S.E.	
Amitriptyline +	5.00			60.0
PBQ	0.62	5/6	953 \pm 126	5.0
Pentobarbital +	20.00			20.0
PBQ	0.62	6/10	304 \pm 89	5.0
Pentobarbital +	10.00			20.0
PBQ	0.62	4/10	703 \pm 112	5.0
Pentobarbital +	2.50			20.0
PBQ	0.62	6/10	971 \pm 86	5.0
Pentobarbital +	10.00			20.0
No Treatment	----	5/8	328 \pm 117	5.0
Haloperidol +	0.32			120.0
PBQ	0.62	5/5	316 \pm 64	5.0
Librium +	10.00			30.0
PBQ	0.62	4/5	366 \pm 35	5.0
Aspirin +	740.00			45.0
PBQ	0.62	2/6	567 \pm 27	5.0
Aspirin +	160.00			45.0
PBQ	0.62	2/6	653 \pm 125	5.0
Aspirin +	40.00			45.0
PBQ	0.62	3/6	692 \pm 80	5.0
Aspirin +	10.00			45.0
PBQ	0.62	4/6	782 \pm 98	5.0
Haloperidol	0.32	1/6	43 \pm 17	120.0

1 Mg/kg of body weight, except for saline, which was given in ml/kg.

2 Proportion choosing the the PBQ lever of all subjects tested.

* Only 3 of the 5 subjects tested at this dose were able to respond.

TABLE 7
Dose Response for PBQ Discrimination

Dose ¹	N	% Responding on Drug Lever	Response Rate ²
0.00	20	0	774 \pm 40
0.16	10	50	830 \pm 78
0.62	20	100	769 \pm 33

1 Mg/kg I.P. 5 minutes prior to test.

2 Mean \pm standard error for a 10 minute response period.

Figure 6
Dose Response for PBQ Discrimination

Either 0, 0.16, or the training dose of 0.62 mg/kg PBQ (doses of PBQ are found on the X axis) was administered I.P. 5 minutes prior to a discrimination test trial. The percent of total subjects tested (20 at 0, 10 at 0.16, and 20 at 0.62) who selected the PBQ-appropriate lever for responding can be found on the Y axis. (■) denotes PBQ lever selection 5 minutes after a saline injection in 20 subjects.

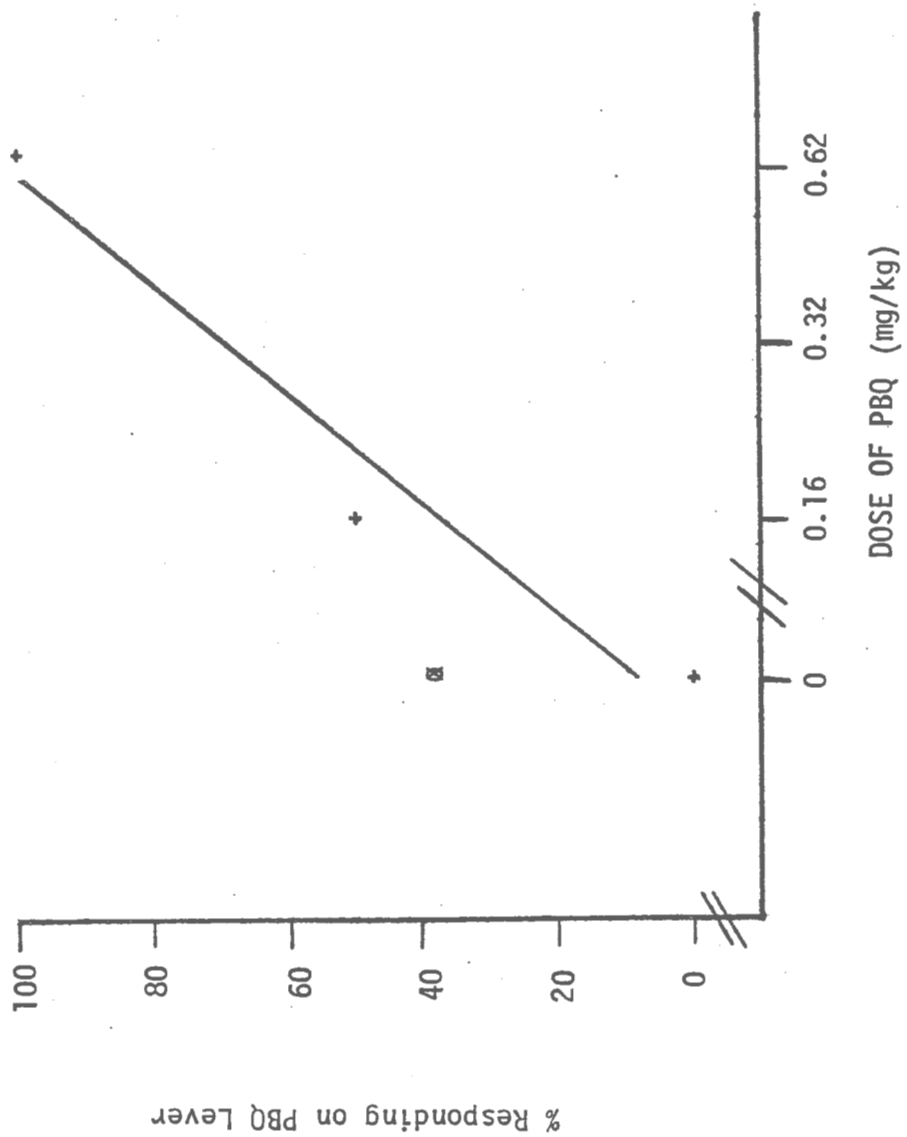


TABLE 8

Tests of Several Treatments for
Generalization with PBQ Stimuli

Treatment	Dose ¹	N	% Responding on Drug Lever	Response Rate
Saline	1.00	20	40	914+ <u>58</u>
Needle Insertion	----	10	50	816+ <u>81</u>
Haloperidol	0.32	6	17	43+ <u>12</u>
Histamine	1.25	6	100	807+ <u>65</u>

1 ML/kg for saline, mg/kg for all others; injections were I.P. for all conditions.

2 Mean ± standard error for a 10 minute response period.

a dose of 0.62 mg/kg PBQ (χ^2 df1=17.1, $P < 0.01$), but was not significantly different from the effects of 0.16 mg/kg PBQ (χ^2 df1=0.27, $P > 0.05$) or needle insertion alone; which produced drug lever responding in 5 of 10 subjects tested. This indicates that a hypodermic needle insertion by itself, can produce significant responding on the PBQ treatment lever, but does not substitute completely for the training dose of PBQ.

Injection of PBQ or saline 30 minutes prior to a test trial produced a decrease in the discriminability of PBQ, but not the saline treatment (see Table 6).

Administration of the antipsychotic, dopaminergic blocking agent haloperidol 2 hours prior to a test trial produced PBQ appropriate responding in only one of six subjects tested (refer to Table 8). This was not significantly different from no-treatment by chi-square analysis (χ^2 df1=3.41, $P > 0.05$), although responding was severely depressed when compared with baseline values for no-treatment (F df1,5=25.81, $P < 0.01$).

Injection of histamine at 1.25 mg/kg to 6 subjects elicited PBQ-treatment responding in all of them, without affecting response rate (t df10=1.22, $P > 0.05$). A summary of these data is presented in Table 8.

Table 9 shows the number of responses made on the non-selected lever for experimental and control tests. These values indicate the mean strength of choice for all

TABLE 9

Strength of Choice as Indicated by the Mean
 Number of Responses on the Non-Selected Lever.
 Shown for Subjects Selecting either the PBQ or
 No-Treatment Lever for Responding after each Test.

Treatment	Dose ¹	PBQ Selecting Subjects ²	Responses on Alternate Lever ³	No-Treatment Selecting Subjects ⁴	Responses on Alternate Lever ²
None	----	0	-----	20	1.6 \pm 0.6
Saline Injection	1.00	8	1.1 \pm 1.0	12	2.7 \pm 0.9
Saline + 30 min.	1.00	6	1.6 \pm 1.2	6	1.2 \pm 0.7
Needle Insertion	----	5	3.8 \pm 2.0	5	4.0 \pm 2.0
PBQ	0.62	20	1.7 \pm 0.6	0	-----
PBQ + 30 min.	0.62	9	2.8 \pm 1.3	3	1.9 \pm 0.9
PBQ	0.16	5	-----	5	3.4 \pm 1.7
Histamine	1.25	6	1.8 \pm 1.0	0	-----
Morphine + PBQ	10.00 0.62	0	-----	5	4.2 \pm 1.8
Morphine + PBQ	5.00 0.62	1	6.0 \pm 6.0	4	1.0 \pm 0.7
Morphine + PBQ	1.25 0.62	4	0.8 \pm 0.8	1	4.0 \pm 4.0
Morphine + Naloxone + PBQ	5.00 1.25 0.62	5	5.4 \pm 2.2	0	-----
Amitriptyline+ PBQ	40.00 0.62	2	1.0 \pm 1.0	1	9.0 \pm 9.0

TABLE 9 Continued

Treatment	Dose ¹	PBQ Selecting Subjects ²	Responses on Alternate Lever ³	No-Treatment Selecting Subjects ⁴	Responses on Alternate Lever ²
Amitriptyline + PBQ	20.00 0.62	5	2.8 <u>±</u> 1.7	1	9.0 <u>±</u> 9.0
Amitriptyline + PBQ	10.00 0.62	3	1.0 <u>±</u> 1.0	3	4.3 <u>±</u> 2.4
Amitriptyline + PBQ	5.00 0.62	5	1.4 <u>±</u> 1.0	1	-----
Pentobarbital + PBQ	20.00 0.62	6	1.0 <u>±</u> 0.5	4	-----
Pentobarbital + PBQ	10.00 0.62	4	0.3 <u>±</u> 0.3	6	0.5 <u>±</u> 0.5
Pentobarbital + PBQ	2.50 0.62	6	3.0 <u>±</u> 1.4	4	7.8 <u>±</u> 0.6
Pentobarbital + NO-Treatment	10.00 -----	3	-----	5	2.4 <u>±</u> 0.9
Haloperidol + PBQ	0.32 0.62	5	4.8 <u>±</u> 1.7	1	7.0 <u>±</u> 7.0
Librium + PBQ	10.00 0.62	4	6.3 <u>±</u> 1.8	1	6.0 <u>±</u> 6.0
Aspirin + PBQ	740.00 0.62	2	0.5 <u>±</u> 0.5	4	2.6 <u>±</u> 1.5
Aspirin + PBQ	160.00 0.62	2	6.0 <u>±</u> 1.0	4	5.3 <u>±</u> 1.9
Aspirin + PBQ	40.00 0.62	3	1.0 <u>±</u> 1.0	3	1.7 <u>±</u> 1.7
Aspirin + PBQ	10.00 0.62	4	4.5 <u>±</u> 2.6	2	-----
Haloperidol	0.32	1	2.0 <u>±</u> 2.0	5	3.5 <u>±</u> 1.2

TABLE 9 Continued

- 1 Mg/kg of body weight, except for saline, which was given in ml/kg.
- 2 Total number of subjects selecting the PBQ lever for responding after the indicated test treatment.
- 3 Mean responses (\pm S.E.) which were emitted on the non-selected lever prior to 10 responses on the other lever.
- 4 Total number of subjects selecting the no-treatment lever for responding after the indicated test treatment.

lever selections. Strength of choice is indicated by the level of responding on the non-selected lever by each subject prior to completion of 10 responses on the selected lever. Low values show that the subjects did not respond haphazardly until obtaining reward, but tended to complete all 10 responses on the lever selected. It is interesting that the values for subjects selecting the PBQ or no-treatment levers on control tests did not differ in this respect. A value of five or larger, however, may be indicative of a significant degree of uncertainty. The single PBQ selecting subject tested at 5.0 mg/kg morphine responded six times on the non - PBQ lever before completing 10 responses on the PBQ treatment lever. A similar result is seen with 20 and 10 mg/kg amitriptyline treatments. Subjects selecting the no-treatment lever responded on the PBQ treatment lever an average of 9 times before completing their choice. Subjects selecting the no-treatment lever after 2.5 mg/kg pentobarbital completed a mean of 7.8 responses on the PBQ treatment lever first. The same pattern can be seen following pretreatment with haloperidol. Also, both librium and 160 mg/kg aspirin treatments produced large numbers of responses on the non-selected lever regardless of the final choice. It is of interest that pentobarbital treatment did not result in large numbers of responses on the non-selected lever, as might be predicted by a state dependent explanation of its

effect on choice behavior. In general, it appears that choice is made in a clear-cut fashion, and not at random.

Effect of analgesics and other centrally acting drugs on discrimination of a PBQ injection

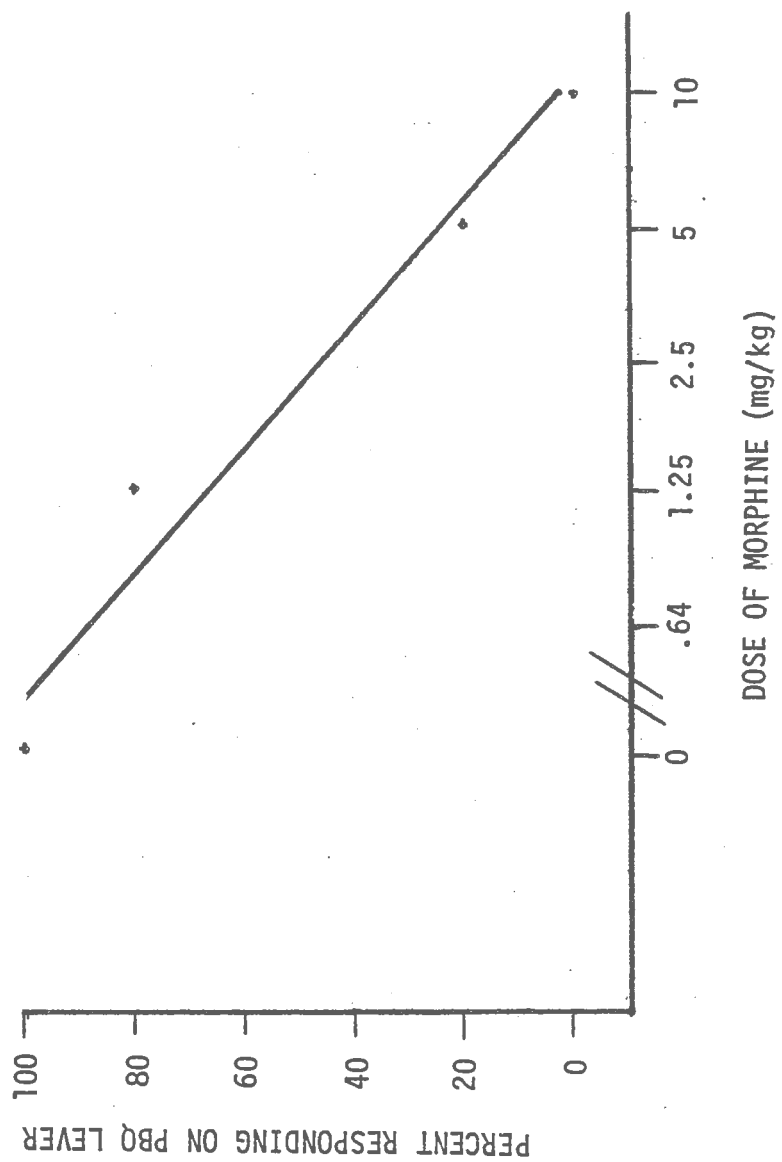
Discrimination of the PBQ injection was observed following pretreatment with either morphine or aspirin on several test trials. A summary of the results can be seen in Table 6. A dose-dependent blockade of PBQ treatment discrimination is evidence that the basis of the discrimination involves both sensory and aversive affective components by which we define pain. The results which follow support this hypothesis.

Morphine sulphate pretreatment prevented discrimination of the PBQ injection in a dose-dependent manner, as shown in Figure 7. Four of the 5 subjects pretreated with 1.25 mg/kg responded on the PBQ injection lever, while only one of the 5 subjects tested at 5 mg/kg did so. When 10 mg/kg morphine was given prior to PBQ treatment, all subjects chose the non-PBQ lever for responding. The correlation coefficient for the computer generated best linear function was 0.955, $F_{df1,2}=20.52$, $P < 0.05$. The ED50 for blockade of PBQ discrimination was 4.06 mg/kg. An analysis of variance indicated a significant effect of morphine on response rate

Figure 7

Effect of Morphine Pretreatment on PBQ Discrimination

Discrimination test trials were carried out 5 minutes after administration of the training dose of 0.62 mg/kg PBQ, following pretreatment 30 minutes earlier with either 0, 1.25, 5.0 or 10 mg/kg morphine sulphate (X axis). The percent of subjects tested at each dose (N = 5 at each dose) who selected the PBQ appropriate lever for responding can be found on the Y axis.



($F_{df3,16}=5.89$, $P < 0.01$), although this effect was non-linear ($r=0.879$, $F_{df1,2}=6.089$, $P > 0.05$). The between groups variance was primarily accounted for by the response suppressing effects of the 10 mg/kg dose as compared with the others by a Tukey followup analysis ($P < 0.01$ for 10 mg/kg vs. all other doses). The 5 mg/kg dose did not affect response rate as compared to no treatment ($P > 0.05$, Tukey test). Morphine in combination with naloxone had no significant effect on the PBQ treatment discrimination, as shown in Table 6.

The blockade of PBQ treatment discrimination by morphine, a narcotic analgesic, suggests that the discrimination is based on a painful experience. This experience includes both sensory and negative affective components. The analgesic action of morphine is considered to be largely due to its effect on the aversive emotional component of the painful experience, suggesting that this an important component contributing to the discrimination.

To further evaluate the effect of commonly accepted analgesics on the PBQ treatment discrimination, aspirin was tested in the same manner as morphine. The five doses of aspirin employed ranged from 0 to 740 mg/kg orally.

A linear regression analysis of the common log transformation of the PBQ-aspirin dose response shows that the aspirin-discrimination relationship was non-linear

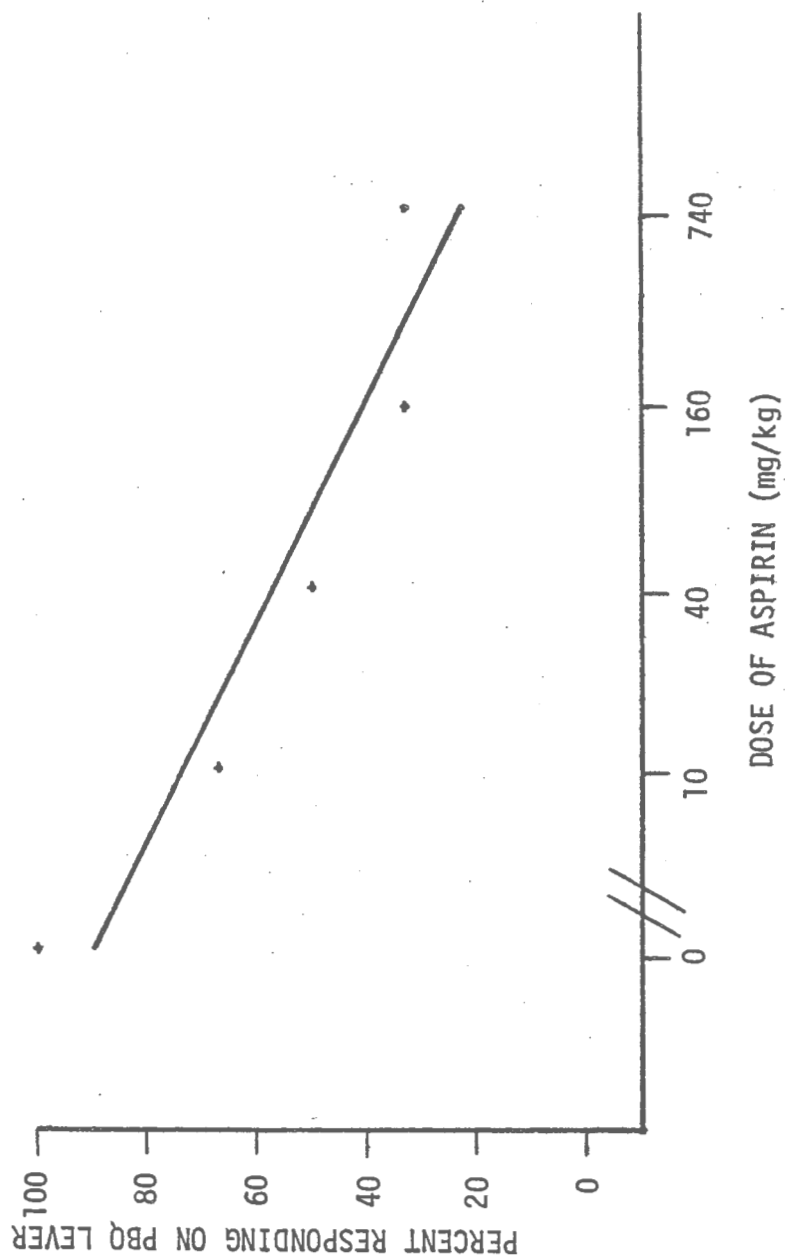
($r=0.70$, $F_{df1,3}=9.58$, $P > 0.05$). The calculated ED50 for aspirin blockade is 340 mg/kg. An analysis of variance of the effect of the doses on response rate shows a significant effect of aspirin ($F_{df3,20}=4.03$, $P < 0.05$). This was due to a significant difference between only two doses, 0 and 740 mg/kg aspirin ($P < 0.01$, Tukey Gap test). As seen in Figure 8, the blockade of PBQ treatment discrimination was not complete, even at the highest dose; unlike the action of morphine. The greatest level of blockade was 67%. This action of aspirin appears to be a central effect, as the drug was administered orally. Doses which have been found to block PBQ-induced writhing differ for different investigators, ranging from 165 mg/kg P. O. (Siegmund et al. 1957), to 750 mg/kg (Evans, 1975). It appears that aspirin raises the threshold for perception of the PBQ treatment, but does not completely suppress it.

The effect of aspirin on PBQ treatment discrimination is further evidence that a central pain experience is the primary basis of the discrimination. However, the question remained after tests with morphine and aspirin whether the blockade of PBQ treatment discrimination is specific to analgesics, or whether other centrally acting drugs may show similar activity. To investigate this possibility several other centrally acting drugs not recognized as possessing analgesic activity were tested in

Figure 8

Effect of Aspirin Pretreatment on PBQ Discrimination

Discrimination test trials were carried out 5 minutes after administration of the training dose of 0.62 mg/kg PBQ following pretreatment 45 minutes earlier with either 0, 10, 40, 160 or 740 mg/kg aspirin given orally to 6 subjects at each dose. Aspirin doses can be found on the X axis. The percent of subjects tested at each dose who selected the PBQ appropriate lever for responding can be found on the Y axis.



the same manner as morphine and aspirin.

Librium, a benzodiazepine muscle relaxant and anti-anxiety drug, did not effect discrimination of the PBQ treatment (χ^2 df3,=1.11, $P > 0.05$) at a dose which severely depressed the subjects' response rates (mean response rate for PBQ alone = 661 ± 100 , and 366 ± 35 for librium pretreatment on PBQ test trials, F df1,8=7.823, $P < 0.05$). For a summary of the data see Table 6.

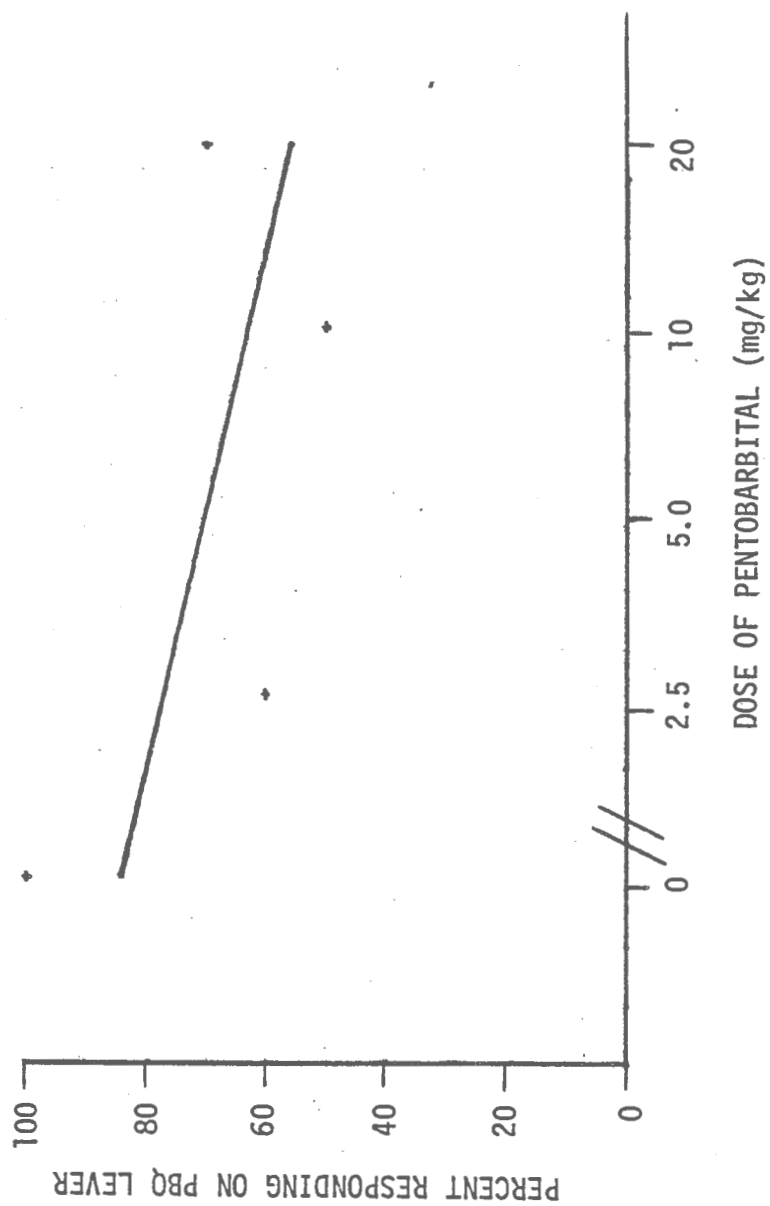
A dose of 0.32 mg/kg haloperidol administered two hours prior to PBQ discrimination tests had no effect on lever selection, as all 5 subjects tested responded on the PBQ treatment appropriate lever. At this dose response rates were suppressed to a mean level of 316 ± 64 from baseline levels of 723 ± 108 , F df1,8=10.588, $P < 0.05$.

Results of pretreatment with pentobarbital are less clear. The dose response curve generated from tests of PBQ treatment discrimination after pretreatment with several doses of pentobarbital was non-linear (F df1,2=9.891, $P > 0.05$), although the correlation coefficient was 0.86. Sixty percent of the ten subjects tested at 2.5 and 20 mg/kg (see Figure 9) responded on the PBQ treatment appropriate lever, and this was significantly different from baseline PBQ discrimination performance of 10 out of 10 correct PBQ choices (χ^2 df1=5.0, $P < 0.05$). At 10 mg/kg pentobarbital 6 of the 10 subjects chose the no treatment lever for reinforcement.

Figure 9

Effect of Pentobarbital Pretreatment on PBQ Discrimination

Discrimination test trials were carried out 5 minutes after administration of the training dose of 0.62 mg/kg PBQ, following pretreatment 20 minutes earlier with either 0, 2.5, 10 or 20 mg/kg pentobarbital given I.P. to 10 subjects at each dose. Doses of pentobarbital can be found on the X axis. The percent of subjects tested at each dose who selected the PBQ appropriate lever for responding can be found on the Y axis.



This is a significant difference from baseline performance of 10 out of 10 on the PBQ appropriate lever ($\chi^2 df1=8.58$, $P < 0.01$). The 20 mg/kg dose of pentobarbital, a sedative-hypnotic central nervous system depressant, caused marked response suppression as seen in a significant analysis of variance ($F df3,36=8.84$, $P < 0.01$). Response rates at 20 mg/kg were significantly different from 0, 2.5, and 10 mg/kg ($P < 0.05$ in all cases by Tukey Gap test).

To clarify the action of pentobarbital on choice behavior, a dose of 10 mg/kg was administered prior to no-treatment trials in 10 subjects at the end of the experiment. If performance accuracy was reduced significantly by this treatment it would provide further evidence for a state dependent explanation of the effect of pentobarbital on choice behavior in general, and not on PBQ discrimination specifically.

The data, shown in Table 6, support this hypothesis. Three of the eight subjects able to respond at this dose (responding was completely suppressed in two subjects) chose the PBQ lever for responding. This is significantly different from baseline no-treatment discriminative performance ($\chi^2 df1=3.94$, $P < 0.05$).

The effect of pentobarbital on PBQ discrimination dose not appear to be an analgesic effect for several reasons. At the highest dose tested response rate

performance was greatly depressed, but over 50% of the subjects tested still chose the PBQ correct lever for responding. If pentobarbital did possess significant analgesic activity it should have caused a greater decrease in PBQ lever selection at less suppressive doses. According to the dose response function, the ED50 value for pentobarbital in this respect would be 18 mg/kg. An effect of pentobarbital on recall of the previously acquired discrimination appears to be a more plausible explanation. Lever selection levels were approximately 50% at all doses of pentobarbital utilized, a value which would be expected if recall of the task were interfered with and lever selection was made at random.

This is an example of a disadvantage of operant behavioral techniques where learning and recall of a previously learned task are essential for valid assay of a drug's effect. Treatments that interfere with memory function, especially recall, may become false positives or negatives if the data are not inspected carefully. In this case an analgesic false positive indication for pentobarbital can be tentatively ruled out since there was not a significant dose-response function which tended to produce complete blockade of PBQ discrimination, even at high response-suppressing doses.

A tricyclic antidepressant, amitriptyline, was tested for effects on PBQ treatment discrimination at four dose

levels; 5, 10, 20, and 40 mg/kg in 6 subjects each. From Figure 10 it can be seen that there was no significant overall dose-response effect on the discrimination ($r=0.65$, $F_{df1,3}=2.19$, $P > 0.05$). Although 50% of the 6 subjects tested at 5 mg/kg responded on the non-PBQ treatment lever ($\chi^2_{df1}=4.0$, $P < 0.05$), this result was not consistent with further tests. At 20 mg/kg all but one of the 6 subjects tested (83%) responded on the PBQ appropriate lever, and at the next higher dose (40 mg/kg) two of the three subjects capable of responding chose the PBQ correct lever. This suggests that the 10 mg/kg result is due to random variation rather than an analgesic or other central nervous system effect of the amitriptyline treatment. The highest dose of amitriptyline reduced response rates significantly compared to all other doses ($P < 0.05$ in all cases by Tukey test, $F_{df4,25}=7.543$, $P < 0.01$), which did not differ between themselves.

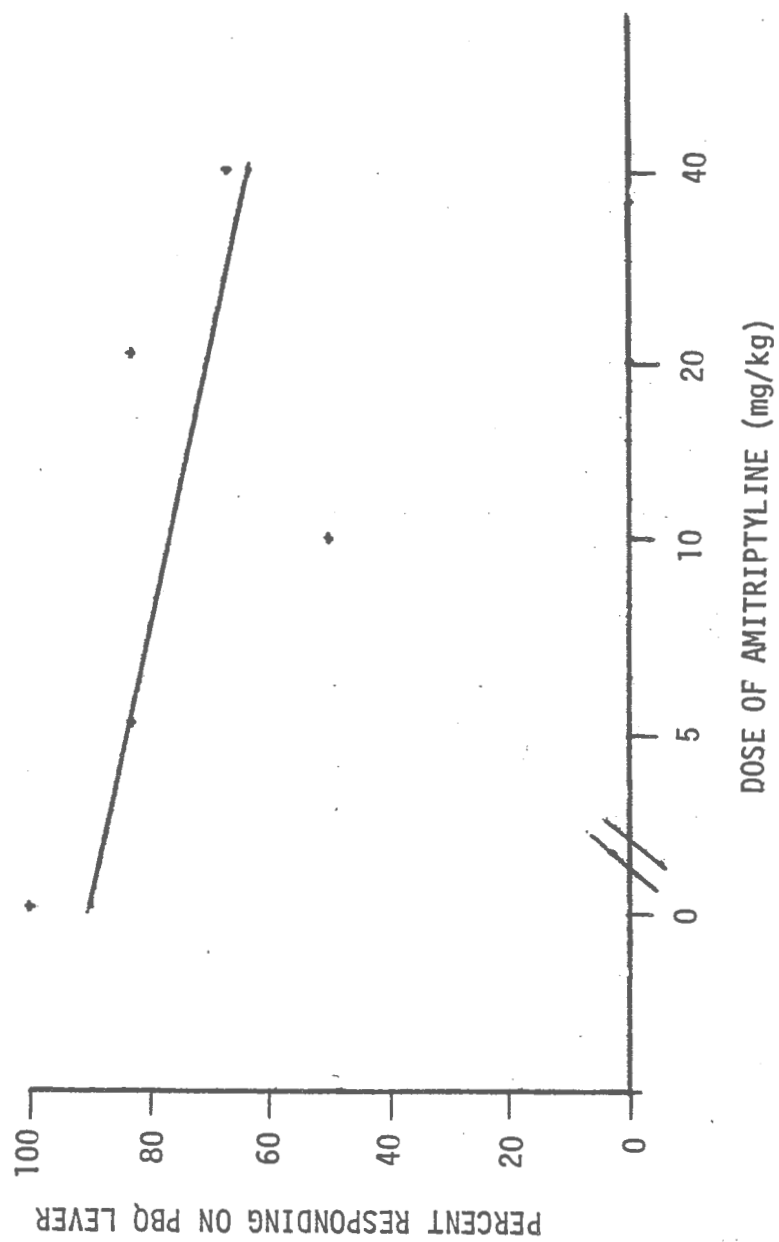
PBQ at 10 mg/kg was administered to four experimental subjects with more than 25 total trials at 0.62 mg/kg PBQ, and produced substantial writhing behavior in all animals. Mean writhes for the four subjects in a 30 minute period were 10.5 ± 5 . This value is not significantly different from that seen in PBQ naive animals chronically treated for six days, as seen in Table 5.

At the end of the experiment an injection of physiological saline produced no writhing behavior in any

Figure 10

Effect of Amitriptyline Pretreatment on PBQ Discrimination

Discrimination test trials were carried out 5 minutes after administration of the training dose of 0.62 mg/kg PBQ, following pretreatment 60 minutes earlier with either 0, 5.0, 10, 20 or 40 mg/kg amitriptyline given I.P. to 6 subjects at each dose. Amitriptyline doses are shown on the X axis. The percent of subjects tested at each dose who selected the PBQ appropriate lever for responding can be found on the Y axis.



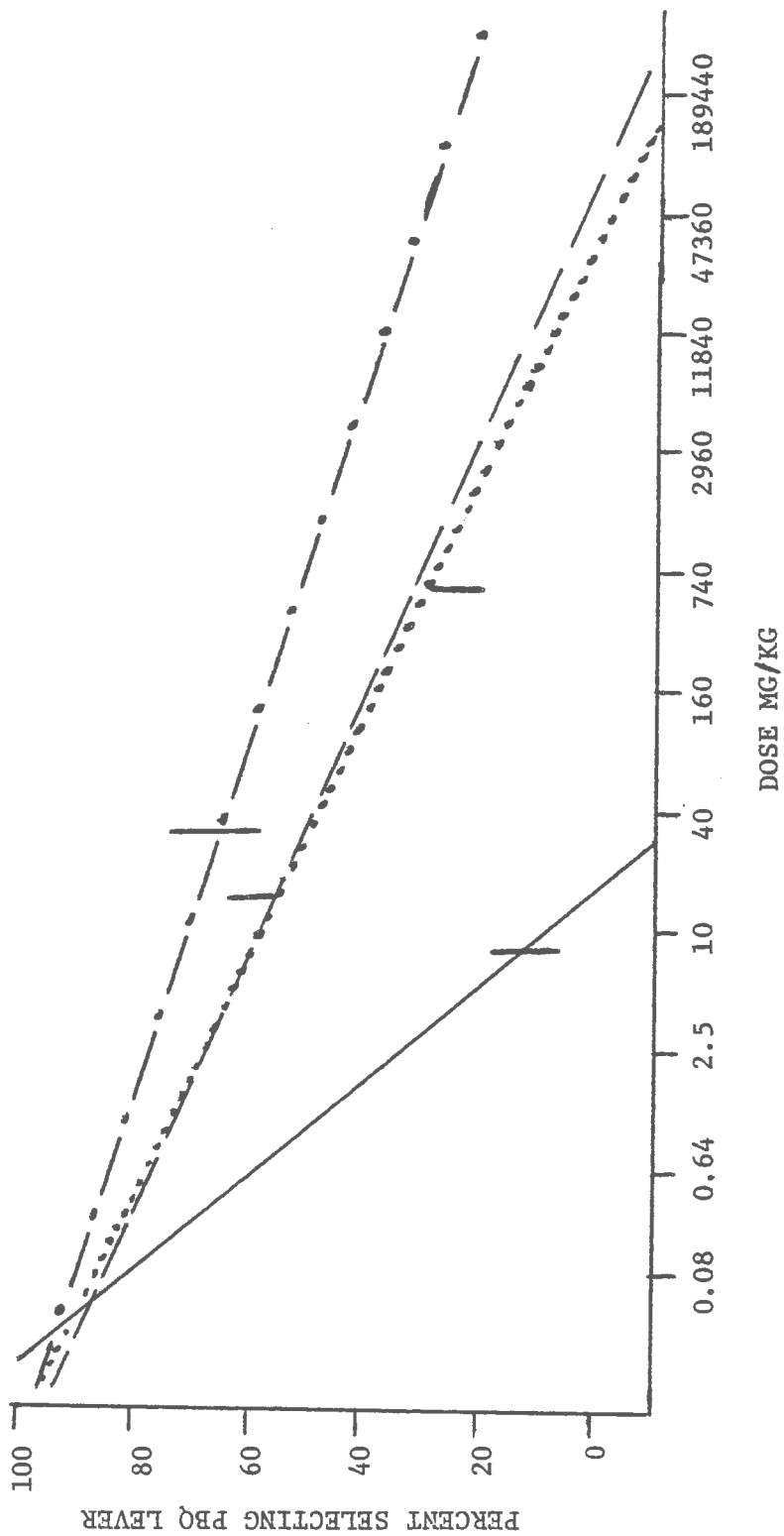
of the subjects tested. A conditional effect of the injection procedure as a component of the discrimination, resulting from repeated PBQ administration during training and testing, appears to be unlikely.

In summary, the data of this section demonstrate that discrimination of the PBQ treatment can be blocked completely by the narcotic analgesic, morphine, and is significantly reduced by aspirin. Although pentobarbital and amitriptyline significantly reduced accuracy of PBQ choice behavior at certain doses, this appears to be due to other effects than analgesia as there were no significant dose response relations between drug treatment and lever selection for these compounds. Also, neither drug was able to reduce PBQ choice to levels produced by either morphine or aspirin, even at doses which severely depressed response rates. The other centrally acting drugs tested, librium and haloperidol, had no significant effects on discrimination of PBQ treatment at doses which significantly depressed response rates. An examination of Figure 11 shows that morphine produces the most potent blockade of PBQ-treatment discrimination with the least response suppression. Pentobarbital and aspirin have similar slopes for PBQ-treatment discrimination blockade, but pentobarbital produces significant response rate decreases at a dose which has little or no blocking action. Any dose further along the the slope for blockade

Figure 11

The Effect of Analgesic and Non-analgesic
Drugs on PBQ Discrimination.

A comparison of the effect of morphine (—), aspirin (·····), pentobarbital (- - - -), and amitriptyline (-·-·-·-) on PBQ treatment discrimination. Vertical Markers (|) indicate the first dose of each drug which produced significant suppression of response rates from baseline. The X axis is dosage in mg/kg of body weight, while the percent of subjects tested at each dose who chose the PBQ lever for responding is shown on the Y axis.



of PBQ-treatment discrimination would be extremely debilitating and possibly lethal, the same is true with amitriptyline.

It was also shown that sensitivity to the writhing inducing action of PBQ was not significantly attenuated by the chronic administration of the training dose during the course of the experiment.

General Discussion

The operant procedure of PBQ treatment discrimination as evaluated by the three experiments included here, is a valid procedure for study of analgesic treatments, but needs some methodological modifications. In Experiment 1, high doses of PBQ produced a reliable writhing response which was blocked by morphine. Since the response is centrally mediated, and may be interpreted as aversive in nature, the analgesic effect of morphine appears to be the primary drug action producing the writhing reduction. The local irritation and accompanying subjective aversive effect together comprise the experience of pain, and morphine blockade of PBQ writhing supports the operational definition of pain; drug-induced writhing blocked by a known analgesic. In this experiment a saline injection did not produce any writhing indicative of pain. To ascertain whether PBQ treatment could serve as a discriminative stimulus, an injection of PBQ was presented prior to availability of food reinforcement from a particular location, and a saline injection preceded availability of reinforcement from a second location over many trials. The differential treatments attained significant control over lever pressing choice behavior for the group of 20 subjects in 70 trials. However, the

task was not maintained accurately. Only 7 of the 20 subjects reached an acceptable performance criterion for demonstration of reliable task learning. This criterion was attained in a relatively large number of trials (49±8) when compared with acquisition of discrimination using a centrally acting drug such as morphine, which is typically learned in fewer than 35 trials (Miksic and Lal, 1977). In addition, the repeated PBQ treatment used for discrimination (1.25 mg/kg) was quite toxic. Only four of the twenty subjects were able to complete more than 90 total trials, although PBQ was not seen to significantly effect response rates as compared to saline treatment. It seemed possible that the PBQ-saline discrimination was in reality a discrimination between two different levels and/or qualities of similar stimulation ("pain"), rather than a pain - no pain discrimination. Modifications in the discrimination training procedure were made when Experiment 3 was initiated. To lower toxic effects of the repeated PBQ administration, the dose used for discrimination was reduced to 0.62 mg/kg; and the saline injection was eliminated on non-PBQ trials to increase the distinctiveness of the two discriminative conditions. These changes did not significantly affect rate of acquisition or reduce toxicity of the PBQ treatment. However, enough subjects were utilized so that a number of experimental manipulations could be applied on

post-criterion trials of subjects who demonstrated consistent performance. These tests indicated that discrimination of the PBQ treatment was dose-dependent and the threshold for discrimination, ED50, was 0.26 mg/kg. Tests of the effects of a saline injection show that the stimuli produced by PBQ were not the only basis of the PBQ treatment discrimination. Forty percent of subjects treated with saline prior to a test trial chose the PBQ treatment lever for responding. The discriminative stimuli produced by the PBQ treatment are therefore not solely due to the action of the PBQ itself, but represent a compound of which the PBQ action is the major component. This does not invalidate PBQ treatment discrimination as a measure of subjective "pain", but the stimuli cannot be described as resulting solely from internally produced visceral effects of PBQ. Although internal PBQ-induced irritation is the primary basis of the discrimination, the irritation produced by the injection procedure is a contributing factor. In order to make the pain inducing stimulus more specific in future experiments, administration could be via an indwelling I. P. cannula.

Further questions about the specificity of the PBQ treatment discriminative stimulus included whether non-specific effects of centrally acting drugs would generalize to the stimulus. To study this possibility haloperidol was administered two hours before a test

trial, when its peak action is in effect and the injection procedure is temporally separated from the test trial. Haloperidol depressed responding to a highly significant degree but had no effect on lever selection. This suggests that intense but non-specific central nervous system effects do not generalize to PBQ discriminative stimuli. Injection of histamine, a substance which produces reports of intense pain when injected in man, resulted in PBQ treatment responding in all subjects tested without affecting response rate. This is further confirmation that the primary quality of the PBQ treatment that supports discriminative choice behavior is a combined sensory-aversive experience, or pain.

Pretreatment with the analgesics morphine and aspirin resulted in significant blockade of the PBQ treatment discrimination, thus demonstrating that the procedure is sensitive to analgesic effects of both narcotics and salicylates. Librium and haloperidol had no detectable effect on PBQ treatment discriminative choice behavior, while severely affecting response rate. Pentobarbital and amitriptyline had no significant dose-response effect on PBQ discrimination, although both drugs did show significant effects at specific doses. The test differentiates between the pentobarbital and amitriptyline effects, and those of analgesics, in that their effect on PBQ discrimination did not approach that produced by the

two analgesics nor did it result in a significant dose-response function for blockade even at doses which significantly depressed responding. The effect of pentobarbital on PBQ choice responding could hypothetically be explained as an effect on memory processes, specifically recall. Such an effect can be distinguished with tests of several doses. If task recall is interfered with, and analgesia does not play a role, choice behavior should be random and not differ significantly from 50% selection of either of the two possible responses.

The advantages of this procedure as a model of subjectively experienced "pain", or aversive stimulation, for evaluation of analgesic treatments include the following; it is based on an objective measure of subjective treatment effects and is thus more representative of human pain processes. Specific analgesic effects, detected via choice behavior, may be distinguished from non-specific excitatory and depressant effects which are reflected in response rates. The procedure can be utilized to investigate the relationship between different kinds of subjectively experienced aversive stimuli.

Improvements in the task are also warranted, and should be investigated before it is accepted as a fully developed model for the study of pain perception. A main

problem is toxicity which appears to interfere with acquisition and maintenance of accurate performance and which necessitates continual training of new subjects to replace those lost during training and testing. PBQ appears to be an undesirable agent to act as the pain stimulus in this model. The procedure could be improved by using a drug or other treatment which produces significant irritation without severe tissue damage when chronically applied. Histamine and bradykinin are likely candidates for this role. Also, treatments which interfere with memory function are possible sources of false positive indications in analgesic tests unless several drug doses are tested and the data carefully inspected.

Summary

The evidence that PBQ treatment discrimination is based on a painful experience (as defined by specific blockade by analgesics, and generalization to the effects of other irritating chemicals) is of theoretical as well as practical importance. It is the first demonstration that laboratory animals will learn a two choice discriminative task based on both physical and aversive qualities of the stimulus. This was indicated by the morphine blockade of PBQ discrimination. Morphine does not primarily act by disrupting sensory input but decreases the emotional component of the experience in human clinical use. If the discrimination had been based only on the discriminability of peripheral sensory cues, morphine treatment would not have had a significant effect on choice performance once the task was acquired. It is interesting that aspirin, an anti-inflammatory agent, was not as effective in reducing discriminability of the PBQ treatment as was morphine, even at very high doses.

It may be concluded that discrimination of the PBQ treatment qualifies as a model of subjectively experienced pain, which may be distinguished from non-specific stimulation or depression. The model can, and should, be utilized with other aversive stimuli as the functional

aversive stimulus to be discriminated.

Practical applications of this procedure include screening of potential analgesic treatments, and investigation of subjective pain processes. An example of the latter would be a task requiring the subject to discriminate between two types of painful stimuli, such as heat and electric shock. This would allow study of differential effects of a single drug or other experimental treatment on two kinds or qualities of aversive stimuli.

Appendix 1

Experiment 3 Test Treatment Schedule

Subject	Order of Test Treatments ¹
1	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 1826 27
2	2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19.....27 1
3	3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20.....1 2 3
4	4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21.....
5	5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21.....
6	6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22...
7	7 8 9 10 11 12 13 14 15 16 17 18 19 20 21.....
8	8 9 10 11 12 13 14 15 16 17 18 19 20 21.....
9	9 10 11 12 13 14 15 16 17 18 19 20 21.....
10	10 11 12 13 14 15 16 17 18 19 20 21.....
11	11 12 13 14 15 16 17 18 19 20 21.....
12	12 13 14 15 16 17 18 19 20 21.....
13	13 14 15 16 17 18 19 20 21.....
14	14 15 16 17 18 19 20 21.....
15	15 16 17 18 19 20 21.....
16	16 17 18 19 20 21.....
17	17 18 19 20 21.....
18	18 19 20 21.....
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26	26 27 1 2.....

1 See Table 6 for treatments 1 through 27 respectively.

This treatment schedule was subject to modification according to the following conditions. If 5 or more observations were sufficient to demonstrate blockade of PBQ discrimination as determined by a significant Chi-square analysis, or lack of blocking action at a response suppressing dose (as determined by analysis of variance with Tukey followup tests and Chi-square analysis), the remaining subjects scheduled for that treatment were used for the next scheduled treatment with the condition that a subject was not utilized for more than one dose of a drug if possible.

A criterion performance level of at least 4 consecutive correct trials (2 PBQ and 2 no-treatment) was required for all subjects prior to any test. Approximately 50% of all pre-test trials were PBQ or saline.

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